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Natural Variation in the Leaf Epicuticular Wax Alkanes and Leaf Wax Morphology of Trembling Aspen (Populus tremuloides Michx.)

by

© BARRY C. JAQUISH

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE
IN
FOREST SCIENCE

EDMONTON, ALBERTA
SPRING, 1981

This thesis is dedicated to Dianne and to the memories of Mr. and Mrs. R.H. Jaquish

THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Natural Variation in the Leaf Epicuticular Wax Alkanes and Leaf Wax Morphology of Trembling Aspen (Populus tremuloides Michx.) submitted by BARRY C. JAQUISH in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in FOREST SCIENCE.

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Date March 25, 1981

Abstract

Gas-liquid chromatography (GLC) was used to qualitatively and quantitatively analyze the hydrocarbon fraction of Populus tremuloides Michx. leaf epicuticular waxes, and to describe the inter- and intraclonal, temporal, sexual and geographic variation in the hydrocarbons. The wax hydrocarbon fraction of leaves collected from long and short-shoots from the four cardinal directions within three crown strata of four ramets from three natural clones was analyzed to describe inter- and intraclonal variability. The hydrocarbon fraction was composed of seven normal-alkanes, with odd-carbon-numbered compounds, particularly heptacosane (nC27), predominating qualitatively and quantitatively. Significant ($p<.05$) differences in alkane percentages existed among clones and crown strata, and between leaf types. A positive relationship was found between both tricosane (nC23) and pentacosane (nC25) and height within crown. The inverse was true for nC27. Broad-sense heritabilities for all of the alkane characters except nC25 were high (>.60), indicating phenotypic differences in the alkane characters are largely due to genetic differences among clones. Individual clones exhibited a unique alkane profile indicating leaf wax alkanes have potential as clone delineators.

Temporal variation in alkane concentrations was described by analyzing the epicuticular wax alkanes of short-shoot leaves collected approximately every two weeks throughout the growing season from a standardized crown position on single ramets of four clones. All alkanes except nC23 and nC25 varied significantly over the growing season. The major transition in alkane concentrations occurred approximately six weeks following leaf flush and may be related to the cessation of foliar surface wax deposition.

Epicuticular waxes from short-shoot leaves collected from a standardized crown position on 3 ramets of 18 staminate and 17 pistillate clones were analyzed to determine if variation existed between staminate and pistillate foliar waxes. Staminate clones contained significantly higher concentrations of nC23 and nC25, and significantly lower concentrations of nC26 and nC27. Discriminant analysis correctly classified 83% of the clones to their appropriate sex. Scanning electron microscopy revealed pistillate foliar wax is composed of dense, cruciform-shaped platey wax crystals which are oriented perpendicular to the leaf surface, while staminate foliar waxes are less dense and are oriented horizontal to the leaf surface. This variability in wax morphology may give the selective advantage to pistillate clones in hotter environments and to staminate clones in cooler environments.

Leaf epicuticular wax alkanes of short-shoot leaves collected from 5 clones from each of 35 Alberta aspen populations were analyzed to elucidate local patterns of geographic variation in the alkanes. Alkane concentrations were significantly correlated with elevation and average growing season daily temperature. Significant differences among populations were found for all alkanes except nonocosane (nC29). Cluster and discriminant analyses of the alkane characters delineated two distinct groups of Alberta aspen. A high elevation group, composed of northern and high elevation populations, contained lower concentrations of nC22,23,24, and 25, and higher concentrations of nC27, relative to the lower elevation central populations.

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I would like to express my sincere appreciation and gratitude to the members of my graduate committee for their assistance and encouragement throughout this study. Bill Cheliak's assitance with field collections is greatly appreciated.

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I. INTRODUCTION

Most aerial structures of terrestrial plants are enveloped by a complex aggregation of lipid materials collectively known as "waxes". These outer, or epicuticular, waxes combine with a cutinaceous layer to form the plant cuticle. The cuticle functions primarily as a waterproofing barrier between the plant epidermis and the atmosphere, although it also protects against mechanical damage, fungi, bacteria, insects, ultraviolet radiation, and air pollutants (Hanover and Reicosky 1971; Mazliak 1968).

The chemical composition and morphology of foliar epicuticular waxes of a number of tree species (primarily _Eucalyptus_) and agronomic species has been well documented (e.g., Chambers _et al_. 1976; Davis 1971; Hall _et al_. 1965; Hallam and Chambers 1970; and Hanover and Reicosky 1971). The conclusions of these studies may be summarized as follows:

1. Leaf epicuticular wax morphology is a function of the wax chemical composition.
2. Chemical compostion and, hence, morphology of leaf epicuticular wax is under genetic control.
3. The morphology of leaf surface waxes is adaptively significant in many plant species. Glaucousness, for example, is the result of vertical orientation of wax deposits, and has been shown to increase frost hardiness, reduce cuticular transpiration, and reduce

leaf temperature by scattering incident solar radiation.

4. Leaf epicuticular wax biochemicals and morphology may serve as chemotaxonomic criteria in many plant taxa.

Information pertaining to leaf surfaces of many North American tree species is extremely limited. Therefore, information about the leaf surfaces of trembling aspen Populus tremuloides Michx.), the most widespread and abundant tree species in North America (Harlow and Harrar 1969; Keays et al. 1974), would be valuable not only in furthering our knowledge of the ecology and genetics of the species, but in expanding our understanding of plant waxes in general.

There were two objectives to the present study. The primary objective was to describe the phenotypic variation in leaf epicuticular wax hydrocarbons of trembling aspen and to describe, via scanning electron microscopy (SEM), the morphology of trembling aspen leaf epicuticular waxes. A secondary objective was to utilize and obtain an appreciation of multivariate statistical analyses in biologically oriented problems.

Initially, background information regarding biochemical analysis of secondary metabolic products and previous plant wax research will be discussed. Secondly, a relatively brief discussion of the autecology of trembling aspen will be included. Intraspecific variability of trembling aspen leaf wax hydrocarbons will be described by subdividing the body of the thesis into four major sub-sections: temporal, inter-

and intraclonal, sexual, and geographic variation. The four sub-sections will be treated as separate experiments. The SEM investigation will describe morphological variation between staminate and pistillate foliar waxes.

A. Background Information

Biochemical Analysis

The disciplines of plant taxonomy, physiology, genecology, and genetics have been enhanced enormously in recent years through the increased appreciation and comprehension of biochemical analyses, and through technological development in both laboratory apparatus and techniques. This technology facilitates rapid qualitative and quantitative biochemical analyses of small samples from a large number of individuals, thus providing a multitude of new traits upon which modern taxonomic, genecological, or genetic investigations may be based. These new traits also may be used to supplement and substantiate current autecological information, which is based primarily upon anatomical or morphological criteria. In the future, many complex and presently unexplained plant physiological functions and processes undoubtedly will be understood through biochemical research.

The two major advantages biochemical characters offer over traditional morphological and anatomical characters are that biochemical compounds are often under more simple genetic control and they are closer to the site of gene action than morphological and anatomical characters (Hanover 1974). Therefore, biochemical characters usually are less subject to environmental modification than morphological or anatomical characters (Hanover 1974). Squillace (1976) indicated that heritabilities of many biochemical compounds, such as monoterpenes, are relatively high. These high heritabilities also suggest reletively strong genetic control over biochemical compounds. As a result of this reduced environmental modification, biochemical analysis has become increasingly popular in plant chemotaxonomic and genetic research.

Historically, plant biochemical investigations directed at taxonomic and genecological problems have concentrated on the secondary metabolic products (e.g., terpenes, steroids, waxes, phenolics, and alkaloids), since these compounds have been shown to vary qualitatively and quantitatively among plant genera, species, and races (Harbourne 1968). Qualitative and quantitative variation exhibited by secondary compounds makes them more favourable than the ubiquitous primary metabolic products (sugars and organic acids) in biochemical studies directed at taxonomy and genecology (Hanover 1974). Despite the apparent benefits of using secondary metabolic products, several inherent problems exist which must be considered when interpreting analyses of these compounds.

Fluck (1963) noted several intrinsic and extrinsic factors that may influence the biochemical constitution of a plant. The intrinsic factors included the individual's genetic constitution; and diurnal, ontogenetic, and seasonal variation. Extrinsic factors included soil and climate. Harbourne (1968) indicated chemical alteration may occur with aging of the sample. Squillace (1976) suggested that individuals often vary greatly both within and among stands in different portions of the range of a tree species. Combined, these factors pose serious problems when using secondary metabolic products to characterize a species biochemically. These factors led Stebbins (1974) to suggest that secondary metabolic products be used as a supplement to morphological characters in plant taxonomic research.

Despite the apparent weaknesses in utilizing many of the secondary metabolic products in biochemical studies, several secondary compounds have demonstrated considerable chemotaxonomic utility. These useful compounds belong primarily to the wax, terpene, and phenolic groups, and have proven beneficial in taxonomy (Mirov _et al_. 1966; Steele and Ronald 1973; von Rudloff 1975); elucidating evolutionary trends (Stransky _et al_.1967); recognizing interspecific hybrids (Hanover and Wilkinson 1969) and polyploid derivatives (Mecklenburg 1966); investigating genetic and geographic variation in natural populations (Hanover 1974; Steele _et al_. 1973); and studying adaptive responses of plant species (Thomas and Barber 1974). Hanover (1974) suggested secondary compounds also may prove useful in the identification of seed sources and as characters used in indirect selection for commercially valuable traits.

In the present study I investigated the chemical compositon and morphology of the leaf surface waxes of trembling aspen Populus tremuloides Michx. These waxes exist as surficial deposits on the plant cuticle and are considered to be secondary metabolic products (Hanover 1974). The particular wax compounds that were investigated were the alkane, or paraffin, hydrocarbons.

Plant Cuticles and Leaf Surface Waxes

In terrestrial plants virtually every aerial organ, with the possible exception of bark and actively growing root structures, is enclosed by a plant cuticle (Hadley 1980; Kolattukudy 1980). The development of a cuticle is thought to be an adaptation to a non-aqueous environment (Caldicott and Eglinton 1973). The cuticle is composed of an outer stratum of surface wax and a continuous cutinaceous substratum (Fig 1). The cutin-wax layer is anchored to the epidermal cells by a pectin layer interspersed with cellulose (Caldicott and Eglinton 1973).

Although the origin of compounds making up the cuticle is unknown, most investigators feel the lipid materials which form the cuticle are synthesized in the edipermal cells and diffuse through the porous epidermal cell wall (Fahn 1974). The thickness of the cuticle is variable among plant species. In general, thicker cuticles are found on sun plants and plants from drier environments (Kramer and

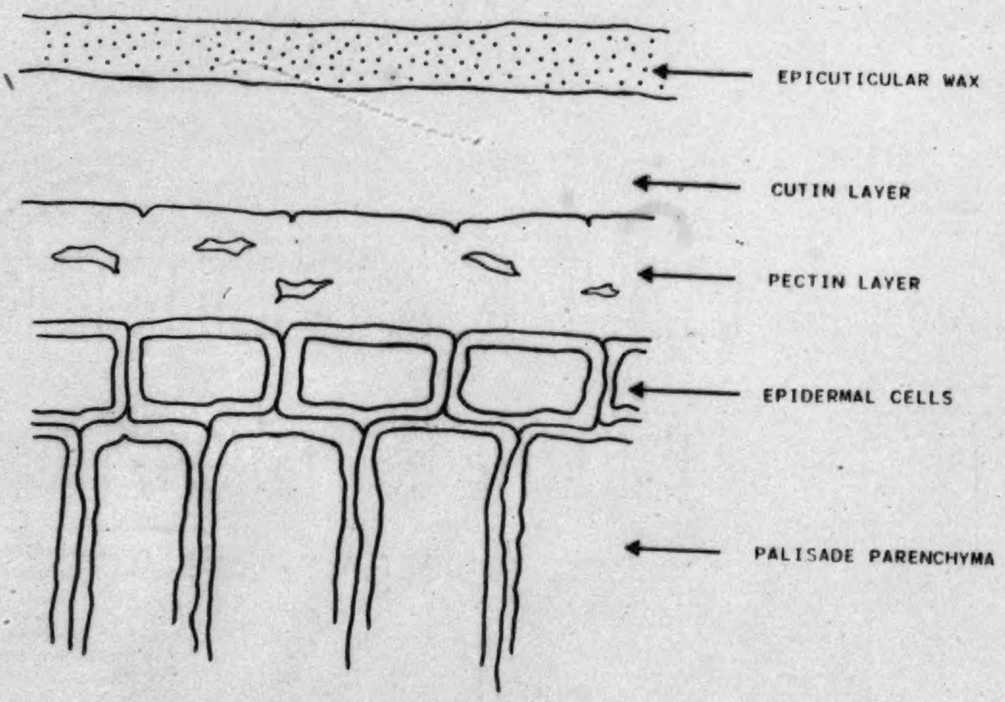

EPICUTICULAR WAX
CUTIN LAYER
PECTIN LAYER
EPIDERMAL CELLS
PALISADE PARENCHYMA

Figure 1 Cross-section of a typical angiosperm leaf cuticle; not drawn to scale. (From Eglinton and Hamilton 1967)

Kozlowski 1979).

The surface, or epicuticular, waxes of interest in this study are believed to be synthesized with the other cuticular materials in the epidermal cells (Kramer and Kozlowski 1979). These wax materials are deposited along the periphery of the plant cuticle and are characterized as being thermally stable (melting point 40-100°c) and hydrophobic (Hadley 1980; Mazliak 1968).

The morphology of the leaf surface waxes of many plant species has been extensively studied by scanning electron microscopy (SEM) (Chambers *et al*. 1976; Davis 1971; Schieferstein and Loomis 1956,1959). These SEM investigations have shown leaf epicuticular waxes to be crystalline structures which exist either as platelets, filaments, tubes, or occasionally homogeneous layers. Wax morphology has been shown to influence the leaf surface characteristics of many plant species. As mentioned previously, glaucousness in many species is attributed to increased light reflectance caused by filamentous or tube surface wax morphology, while non-glaucousness typically indicates a flat, platey wax structure (Hall *et al*. 1965; Reicosky and Hanover 1978). Although the mechanisms responsible for the variation in leaf wax morphology remain unknown, two theories attempting to explain the variation have been proposed (Chambers *et al*. 1976; Hallam 1970; Martin and Juniper 1970). Both theories suggest morphology is affected primarily by the manner in which the wax reaches the leaf surface. Hall and Donaldson (1962) observed pores and channels through the cuticle of *Eucalyptus* leaves and suggested surface waxes were secreted through these passages. This led Martin and Juniper (1970) to suggest that leaf surface wax morphology may be a function of the morphology of these passages. Alternatively, Chambers *et al*. (1976) and Hallam (1970) recrystallized waxes extracted from *Eucalyptus* leaves from a variety of solvents and compared the morphology of the recrystallized wax to natural leaf wax of the same species. In both reports, morphology of the two waxes was similar; leaf epicuticular wax morphology was concluded to be a function of the chemical composition of the wax.

Biochemical analyses have supported the proposal that wax morphology is a function of chemical composition. In *Poa colensoi* Hook.f. and *Eucalyptus urnigera* Hook.f., glaucous phenotypes possessing filamentous surface wax crystals contained high concentrations of *B*-diketones and low concentrations of hydrocarbons, while nonglaucous phenotypes possessing platey surface waxes contained high concentrations of hydrocarbons and low concentrations of *B*-diketones (Hall *et al*. 1965; Thomas and Barber 1974). Chambers *et al*. (1976) reported similar results in *Eucalyptus globulus* Labill. and *E*. *saligna* Sm. The filamentous leaf wax of *E*. *globulus* was charaterized by a high ketone content, while the platey wax of *E*. *saligna* was characterized by a high hydrocarbon content. These results indicate that surface wax morphology is a function of chemical composition, and that considerable interspecific variability in leaf epicuticular wax morphology exists.

The primary physiological function of epicuticular wax is to control water permeability in the cuticle (Caldicott and Eglinton 1973; Eglinton and Hamilton 1967; Kramer and Kozlowski 1979). Several authors maintain that surface wax morphology is important as a light-scattering agent of the leaf, thus reducing undesirable temperature buildup within the leaf (Hall et al. 1965; Reicosky and Hanover 1978). In several species of Eucalyptus and Triticum, surface waxes have been shown to influence frost hardiness (Thomas and Barber 1974; Tulloch 1976). Surface wax also affects the wettability of leaves; therefore, considerable interest regarding wax structure and development has been generated among agronomists concerned with crop spraying (Caldicott and Eglinton 1973). Cameron (1970) reported that the morphology and quantity of Eucalyptus foliar surface wax affected light absorption in the 400-700 nm waveband. Glaucous leaves reflected more incident light than non-glaucous leaves and, consequently, had lower rates of photosynthesis.

Surface waxes are easily extracted by submerging the leaf in an organic solvent (Mazliak 1968). Chemical analysis of the extracted wax is readily accomplished through thin-layer chromatography and gas-liquid chromatography (Tulloch 1976). The results of these types of analyses have indicated that leaf surface waxes are composed primarily of alkanes, alchohols, acids, aldehydes, acetals, and ketones (Eglinton and Hamilton 1967), although small portions of di-and tri-substituted derivatives, plus aromatic phenolic compounds, are occassionally present (Caldicott and Eglinton 1973).

Inter- and intraspecific variation has been reported in the chemical consitution of surface waxes. Eglinton and Hamilton (1967) reported the alkane proportion of sugar cane (*Saccharum officinarum* L.) wax to be approximately 10 percent, while the alkane proportion of *Cotyledon orbicularis* L. was approximately 100 percent. Herbin and Robins (1969) found the alkane proportion of leaf surface waxes in many *Eucalyptus* and *Pinus* species to be a fraction of one percent, while they made up more than 90 percent in *Solandra grandiflora* SW. Hall *et al*. (1965) examined intraspecific biochemical variation in *Eucalytus urnigera* leaf waxes and found the concentration of *B*-diketone varied from less than 10 percent to approximately 50 percent.

Since plant waxes are relatively stable secondary metabolic products, substantial biochemical research has been conducted utilizing wax compounds in chemotaxonomic investigations (e.g. Herbin and Robins 1969; Mecklenburg 1966). A limited number of studies concentrated on surface wax development and morphogenesis (e.g. Chambers *et al*. 1976; Hallam 1970). To date, leaf wax research has concentrated on a limited number of plant species. Tulloch

(1976) noted that leaf waxes from only 367 species have been investigated biochemically. The majority of these investigations have dealt with the easily isolated alkanes.

Alkane Hydrocarbons

The alkanes are the simplest organic compounds. They contain no functional groups, since all of the carbon atoms are singly bonded to other carbon or hydrogen atoms. Under ordinary laboratory conditions the alkanes are relatively unreactive (Griffin 1969). They typically exist as either straight-chain hydrocarbons (normal alkanes) or branched hydrocarbons (isoalkanes) and are probably present in varying quantities in all plant waxes (Tulloch 1976). Hadley (1980) indicated that surface waxes composed primarily of normal alkanes are thermally stable and provide maximum water impermeability.

Chemotaxonomic Studies Using Leaf Surface Wax Alkanes

Eglinton *et al*. (1962a) used leaf wax alkanes to investigate the interrelationships of the Crassulaceae endemic to the Canary Islands. Their results revealed variation in alkane carbon number patterns among genera and species. There was, however, only a rough correspondence between the chemical and botanical classification. These somewhat favourable results inspired a great deal of alkane chemotaxonomic research among a wide variety of diverse plant taxa. The results of these studies indicated the alkanes could serve as useful discriminators in many species, while in others their discriminating utility was poor.

Eglinton et al. (1962b) found several species of New Zealand Hebe and Gaultheria could be readily identified by their alkane distribution pattern. However, two varieties of Phormium tenax Forst. could not be distinguished by their alkane distribution pattern. Mold et al. (1963) also could not discriminate among three tobacco (Nicotiana tabacum L.) varieties on the basis of leaf wax alkanes. Alternatively, Hill and Mattick (1966) found differences in alkane distribution between two varieties of Brassica oleracea L.

Mecklenburg (1966) used alkanes from inflorescence surface wax in a chemotaxonomic study of 42 species of potato (Solanum L.). Her results tended to confirm the accepted divisions within the genus. In addition, polyploid species were distinguishable from diploid species on the basis of alkane patterns.

Leaf wax alkane hydrocarbons of plants from different evolutionary levels were studied by Stransky et al. (1967). Qualitative and quantitative differences in alkane concentrations were found between higher and lower plants. Several species demonstrated seasonal and within plant variation in alkane profiles. Quantitative differences were found among the leaf wax alkanes of dormant Picea excelsa Lam. individuals from three distinct populations in Czechoslovakia. Herbin and Robins (1969) suggested leaf wax alkane concentrations were under genetic control; therefore, genetic differences among the *Picea excelsa* populations maybe responsible for the alkane variation. This concept will be elaborated upon in the subsequent discussion of wax development and morphology.

Borges del Castillo *et al*. (1967) found the leaf wax alkanes to be relatively accurate species discriminators in 14 gymnosperm genera native to New Zealand. Martin-Smith and Subramanian (1967) reported a difference in the leaf wax alkane distribution between New Zealand and South American species of *Cortaderia*. Leaf wax alkanes have also been used successfully to identify putative hybrids between *Cupressus arizonica* Greene and *C*. *lusitanica* Mill., and *C*. *lusitanica* and *C*. *macrocarpa* Hartn. (Dyson and Herbin 1968). Herbin and Robins (1968a; 1968b; and 1968c) reported considerable diversity among leaf wax alkane patterns in several species of *Aloe*, *Agave*, and *Eucalyptus*. Within these genera several species were characterized by specific alkane patterns. The alkanes generally were not good discriminators among genera of the Cupressaceae or Pinaceae, although distinct alkane differences were found between the two families.

From these reports it is evident that plant species typically exhibit a characteristic alkane distribution pattern. In many species this pattern is sufficiently unique to serve as a taxonomic criterion; however, in several species the alkane distributions are so similar that species discrimination, based solely on alkane critieria, is difficult. Within-plant and intraspecific alkane variation contribute to the reduced effectiveness of characterizing many plant species. In those species and varieties where the alkanes may be successfully used as taxonomic criteria, the surface waxes typically contain a relatively high concentration of alkane hydrocarbons (Tulloch 1976).

Evidence of Genetic Control of Wax Morphology and Chemical Composition

Barber (1955) found that a positive relationship existed between glaucousness and elevation in eight species of Eucalyptus. Green-leafed phenotypes were found in sheltered, low elevational areas, whereas glaucous-leafed phenotypes predominated on exposed higher elevational sites. Barber concluded this clinal pattern of variation was an adaptive response to increased frost problems at higher elevations. He suggested glaucousness was simply inherited and that there were at least two loci controlling wax development.

In an extension of this work, Barber and Jackson (1957) recorded the frequency of green and glaucous phenotypes in natural populations of Eucalyptus urnigera and in open-pollinated progeny from mothers of known source elevation reared in a common garden. Clinal variation was found in the adult population. In the common garden, 12 of 28 green parents were segregating glaucous seedlings, while 17 of 39 glaucous parents segregated green seedlings.

Natural selection was concluded to be maintaining a cline of genes controlling intensity and pattern of surface waxes.

Hall *et al*. (1965) used eight year old *Eucalyptus urnigera* seedlings from the green and glaucous parents reported by Barber and Jackson to determine if the quantity and chemical composition of leaf wax was altered by gene action. Wax yields from fresh glaucous and non-glaucous leaves were .36 and .30 percent of fresh weight, respectively. While quantitative differences were not apparent, there was a difference in chemical composition between the two leaf types. Wax of the non-glaucous phenotypes contained about 25 percent alkanes, with nC27 being predominant. Alternatively, wax of the glaucous phenotypes contained approximately three percent alkanes, with nC29 being the predominant alkane. Major differences also were found in the *B*-diketones fraction of the waxes. It was suggested that variation in chemical compostion was responsible for the morphological differences and that several genes determined the chemical composition of surface waxes.

Daly (1964) found clinal variation in the degree of glaucousness in *Poa colensoi* between xeric and hydric sites. As in *Eucalyptus unigera*, glaucous individuals predominated on dry sites. No examination of wax morphology was made. Daly proposed that the glaucous individuals had an adaptive advantage in habitats subject to drought.

Von Wettstein-Knowles (1972) estimated that mutations in at least nine gene loci affect the synthesis of *B*-diketones and hydroxy-*B*-diketones in barley. Chemical analyses and morphological examinations were conducted on the wax from mutants of five of these nine loci. Differences in both chemical composition and wax morpholoty existed. It was concluded that *B*-diketone and hydroxy-*B*-diketone synthesis and, consequently, morphology of barley surface wax are under genetic control.

From these reports it is evident that leaf surface wax chemical composition and morphology are strongly influenced by gene action. Therefore, the wax compounds may be useful in plant genetic, genecological, or ecological investigations. The present study will investigate natural variation in chemical compsition and morphology of the leaf surface waxes of trembling aspen (*Populus tremuloides* Michx.).

B. Autecology of Trembling Aspen (*Populus tremuloides* Michx.)

Distribution and Uses

The genus *Populus* L. consists of approximately 30 deciduous tree species whose distributions are restricted primarily to the cooler regions of the northern hemisphere (Anonymous 1965). Based on morphological criteria, the genus typically is subdivided into the following five taxonomically distinct sections: *Turanga* Bge, *Aigeiros* Duby, Tacamahaca Spach., Leucoides Spach., and Leuce Duby (Anonymous 1974). Populus tremuloides belongs to the section Leuce (Harlow and Harrar 1969).

In North America the genus is represented by approximately 10 species of which only a small number reach commercial size or quantity (Harlow and Harrar 1969). Of these 10 native species, trembling aspen is by far the most prevalent (Maini 1968). In fact, trembling aspen occupies the largest natural range and is one of the most abundent tree species in North America (Harlow and Harrar 1969; Keays et al. 1974). The natural range of trembling aspen extends across the Boreal forest, throughout the Great Lakes area, and south throughout the western mountain ranges to approximately 30 degrees N latitude (Fig. 2).

Ecology

Trembling aspen is a very intolerant, aggressive, pioneer species that readily colonizes disturbed areas. It is a relatively short-lived, rapidly growing tree species, that attains an average height of 15 - 18 metres (Harlow and Harrar 1969). In western North America trembling aspen reaches its maximum size (approximately 30 metres) in the Boreal forest between 55 and 56 degrees north latitude (Maini 1968).

Throughout its vast native range trembling aspen inhabits a variety of sites and is subject to extremely variable climatic conditions. Although trembling aspen

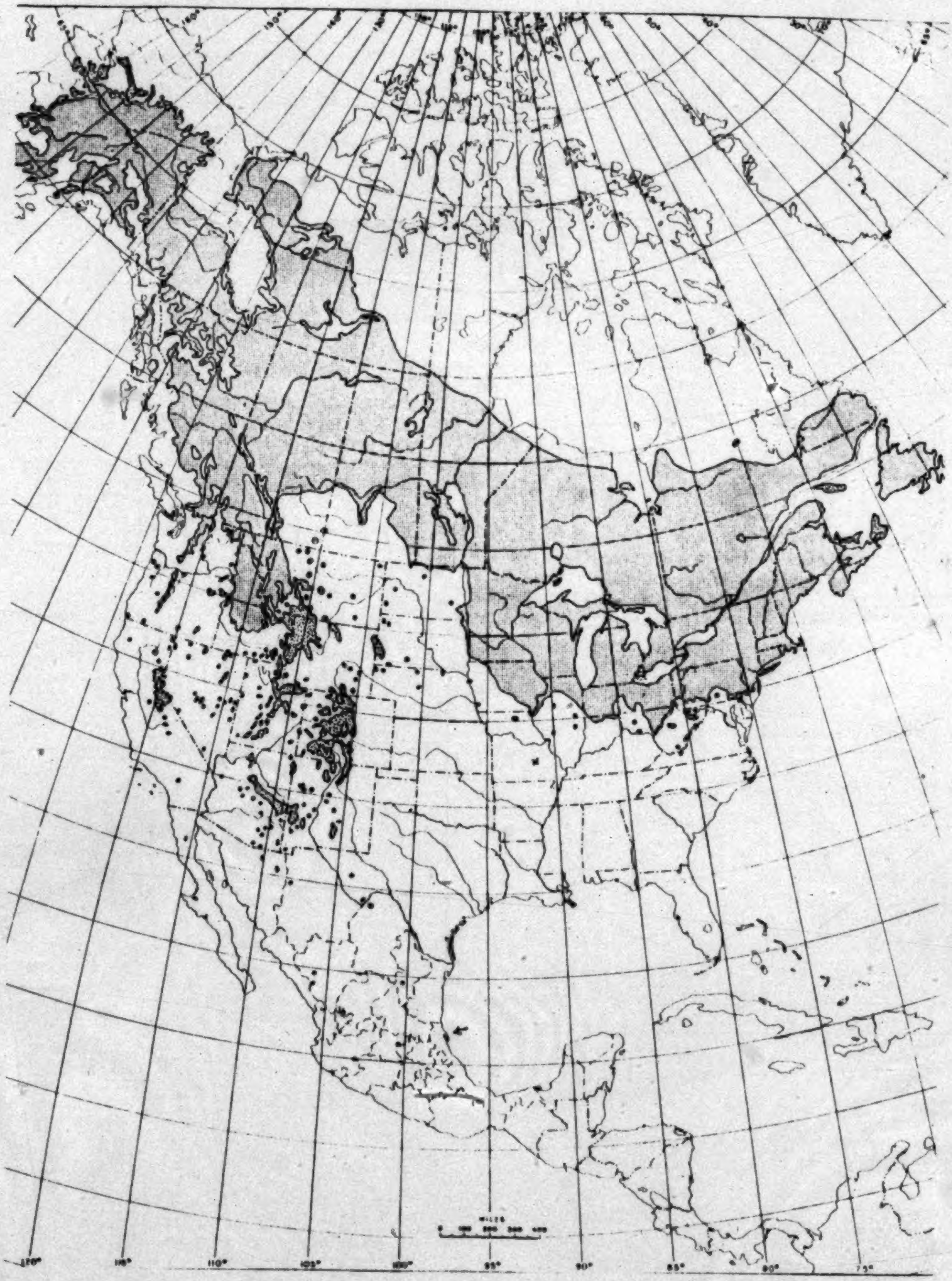

120°
115°
110°
105°
100°
95°
90°
85°
80°
75°
MILES

Figure 2 The natural range of trembling aspen (*Populus tremuloides* Michx.). (From Fowells 1965)

exists on a variety of soil types, optimum growth and development is on porous, loamy, humic, and lime-rich soils (Fowells 1965). Tobiessen and Kana (1974) suggested that trembling aspen in the east is restricted to relatively moist sites, since the species exhibits sluggish stomatal closure under drought stress. In mountainous areas of western North America, aspen typically inhabits areas with cool, relatively dry summers, and abundant winter snowfall (Mueggler 1976).

The number of woody and non-woody plant associates of trembling aspen throughout its native range is extremely large. Fowells (1965) indicated trembling aspen is the major component of six North American forest associations and a minor component of twenty-seven.

Sexual Reproduction

Trembling aspen is typically a dioecious species; that is, staminate (male) and pistillate (female) flowers are typically borne on separate trees (Harlow and Harrar 1969). Deviation from this characteristic sexual expression has been reported. Lester (1963 a) reported inflorescences of 52 of 138 aspen stems from Connecticut displayed some form of bisexuality. The majority of these discrepancies were inflorescences containing both pistillate and staminate flowers.

The floral cycle in trembling aspen is completed within one year (Lester 1963 b). Seeds are generally less than 1 mm long, nondormant, and dispersed by wind approximately three-four weeks following anthesis. Under optimum conditions, seed germination is rapid (1-2 days), germination rates are high (95 percent), and seed remains viable for approximately two to three weeks (Einspahr and Winton 1976). Despite enormous annual seed production, the success rate of seedling establishment is extremely low (Barnes 1969; Maini 1968). Individual seedlings or seedling populations of trembling aspen are sufficiently rare to warrant documentation. (Andrejak and Barnes 1969; Ellison 1943; Larson 1944). A. Gordon (pers. comm. 1980) indicated that seedling establishment north of the Great Lakes is not uncommon.

Poor seedling establishment is attributed primarily to lack of seedbed moisture, low seed viability, and high susceptibility of seedlings to fungal attack (Maini 1972). Adequate seedbed moisture is the most critical factor for the successful sexual reproduction of aspen (Schopmeyer 1974). Since the seedbed must be water saturated for at least one month following seed dispersal for successful germination and seedling establishment, seedling establishment is usually restricted to moist, freshly exposed mineral soil.

Asexual Reproduction

The primary mode of reproduction in trembling aspen is asexual, by suckers from lateral roots near the soil surface

(Barnes 1966). This results in a predominantly clonal growth habit for the species. The clonal growth habit, or reproductive strategy, is common among plant genera (Barnes 1966). In Populus all sections have developed a vigorous clonal growth habit with the exception of Aigeiros and Leucoides (Zsuffa 1975).

The clone, barring somatic mutation, is comprised of a large number of genetically identical individuals (ramets). Ramets originate as suckers from the original seedling plant (ortet) (Barnes 1966). Anatomically, suckers are adventitious shoots originating from newly initiated meristems, pre-existing primordia, or suppressed short shoots in the periderm of lateral roots (Schier 1973; Schier and Campbell 1976). The stimulation and development of root suckers is controlled primarily by apical dominance of the ortet (Maini 1972).

Throughout the growing season auxins produced in the stem are translocated to the root system where they inhibit sucker development (Schier 1976). Surficial disturbances such as fire or logging destroy the ortet, and apical dominance is removed. This leads to a decreased ratio of auxin/growth promotor in the roots, adventitious shoot stimulation, and large numbers of suckers being produced (Schier 1976). Steneker (1976) noted 200,000 aspen suckers per hectare following disturbance; however, heavy competition among the young suckers resulted in high juvenile mortality.

Carbohydrate reserves in the ortet root system supply energy to the newly arisen suckers until individual root systems are generated. Typically, the root system of the ramets will separate, although clones have been reported with interconnected root systems (Tew _et al_. 1969).

This asexual mode of reproduction provides the aspen clone with the capacity to continuously expand and perpetuate itself. Kemperman and Barnes (1976) surmised that many aspen clones in unglaciated regions of the central and southern Rocky Mountains originated as seedlings more than 10,000 years ago. Two of these ancient aspen clones occupied 10.1 and 43.3 ha, while others were estimated to occupy more than 80 ha. Blake (1964) speculated that large clones in Minnesota are of late Pleistocene origin.

Since the clone is the basic genotypic unit of the species, aspen forests typically exist as a mosaic of intermingled multi-stemmed clones of varying shapes and sizes. Investigative work in such forests requires consideration of the clonal habit (Barnes 1966), and clone size must be viewed as a primary component of stand structure (Kemperman and Barnes 1976).

Average clone size has been found to vary among geographic regions. Barnes (1966) reported the average clone size on two sites in Michigan to be .03 and .02 ha. Steneker (1973) estimated the average clone size in two areas of Manitoba to be .08 and .006 ha. Lehn (1979) found the average size of clones in west-central Alberta to be .04 ha.

These estimates indicate a marked difference in clone size between northern aspen populations and populations south of the glacial boundary. These discrepancies in clone size may be attributed to the age of the clones, the density of competing seedlings originally established, the nature and periodicity of disturbances, degree of competition with other vegetation, and site characteristics.

Vigorous asexual reproduction enables trembling aspen to rapidly occupy disturbed sites. This reproductive strategy fully complements the species' ability to reproduce sexually. Sexual reproduction provides recombination of genetic material, which generally is considered advantageous for a species. Seed produced through sexual reproduction also provides the species with long distance dispersal ability. Alternatively, the vigorous asexual capacity enables the species to invade and occupy sites for extremely long periods. The broad geographic distribution and variety of sites occupied by trembling aspen indicate this reproductive strategy is extremely successful.

Aspen's ability to reproduce asexually has stimulated considerable interest among researchers, foresters, and horticulturists. Enormous quantities of information have been obtained regarding the production of asexually reproduced aspen propagules. Specific areas of investigation include numbers of suckers produced (Barry and Sachs 1968; Maini and Horton 1966; Schier 1973, 1975, 1978; Schier and Campbell 1978; Schier and Zasada 1973; Starr 1971; and Tew

1970), biochemical assays of suckers (Hicks 1972), rooting of suckers (Farmer 1963; Zufa 1971), vigor and survival of suckers (Perala 1978), and techniques for large scale production (Benson and Schwalback 1970; Schier and Campbell 1978).

While propagules reproduced asexually from root suckers are obtained with ease, asexual reproduction of aspen from mature stem cuttings is extremely difficult. Success rates in rooting mature aspen stem cuttings usually are less than 10 percent (Barry and Sachs 1968; Khalil 1979; Snow 1938; Stairs and Jeffers 1967). Asexual reproduction by tissue culture of diploid and triploid aspen has been investigated with varying degrees of success (Mathes 1964; Winton 1968a, 1968b, 1970; Wolter 1968).

Sex Ratios

In general, natural populations of sexual organisms have a tendency to maintain an adult male to female ratio of one (Fisher 1930), although in many species, deviations from the expected ratio exist (Bawa and Opler 1977; Freeman et al. 1976; Putwain and Harper 1972). These deviations primarily occur in temperate dioecious plant species (Grant and Mitton 1979; Opler and Bawa 1978), and may occur over very short distances (Freeman et al. 1976). Emperical studies of sex ratios in trembling aspen have indicated considerable variation among geographic regions. Pauley and Mennel (1957) reported a ratio of three male clones to one female clone in Minnesota. Einspahr (1962) found an equal number of staminate and pistillate trembling aspen clones on test sites in Wisconsin. In Colorado the overall ratio was near unity; however, significant deviations from the expected ratio existed if the region was stratified by elevation (Grant and Mitton 1979). Female clones predominated at lower elevations where moisture deficiencies are rare, while males predominated in the higher, drier regions.

This pattern of spatial segregation and sex ratio bias appears to be common among dioecious plant species. Il'in (1973) and Freeman *et al*. (1976) reported sex ratios biased towards females on moist sites and males on dry sites in *Populus tremula* L. and five temperate dioecious plant taxa, respectively. Bawa and Opler (1977), however, found no sexual spatial segregation in four tropical dioecious plant taxa and attributed this lack of sexual sorting to the lack of a distinct moisture gradient. Opler and Bawa (1978) extended their investigation of spatial segregation in tropical dioecious trees and found that only 10 out of 23 species showed a deviation from the unity ratio.

Maynard-Smith (1978) suggested the ecological explanation for sexual spatial segregation in heterogeneous environments may be that female fecundity primarily depends upon the prolonged growing period found on moist sites. Alternatively, plants dispersing pollen would be better adapted to occupy drier sites. The biological mechanisms responsible for sex ratio bias and sexual spatial segregation in plants, however, are not easily explained. Maynard-Smith proposed two mechanisms that may account for sexual spatial segregation: differential mortality and environmentally-determined sexuality. Opler and Bawa (1978) included differential attainment of reproductive status, gamete selection, mode of pollination, and inherent variation in apomictic ability of both sexes as biological mechanisms that may be responsible for sexual spatial segregation. Although studies dealing with differential mortality are lacking, environmentally-determined sexuality was elaborated upon by Charnov and Bull (1977). They indicated that environmentally determined sexuality is not uncommon among plants and animals. Cycnoches and Catasetum were cited as examples of plant genera where femaleness is associated with bright sun and maleness is associated with shade. The suggestion was put forth that environmentally determined sexuality is favoured by natural selection when an individual's fitness, as a male or female, is strongly influenced by environmental conditions and where the individual has little control over which environment it will experience. These reports indicate that both sunlight and moisture may influence plant sexuality.

In trembling aspen, information regarding the physiological and ecological basis for differential mortality rates is lacking. Net photosynthetic and respiration rates, factors that may contribute to differential levels of mortality and fitness, have been found to differ between the sexes (Bordeau 1958). The occurrence of sex chromosomes in aspen has been reported, but not confirmed (van Buijtenen and Einspahr 1959). Therefore, it is difficult to speculate whether gender in aspen is genetically or environmentally determined. Information pertaining to differential attainment of reproductive status, gamete selection, and variation in apomictic ability in aspen is lacking. Mitton and Grant (1980), finding significant differences between aspen sexes in phosphohexose isomerase allele frequencies, suggested the sexes are responding differentially to selection forces and that natural selection, therefore, may be responsible for sexual spatial segregation in trembling aspen.

Genetics

Trembling aspen typically exists as a diploid organism, with a haploid chromosome number of 19 (Smith 1943). This relatively large chromosome number led Stebbins (1974) to suggest the species is of polyploid origin. Naturally occurring triploid (3n=57) clones have been reported, but their frequency is extremely low (van Buijtenen *et al*. 1957; Every and Wiens 1971). Every and Wiens (1971) also reported the existence of a single naturally-occurring tetraploid (4n=76) aspen clone in Colorado.

Interspecific hybridization in *Populus* L. has received considerable attention in recent years. Although hybridization may be used to reveal relationships and evolutionary trends, artificial hybridization in Populus L. has concentrated on discovering parents whose progeny exhibit heterosis, maximum response to intensive sivicultural practices, disease resistance, improved wood qualities, or ease of of propagation (Benson 1972). Trembling aspen has been one of the members of many of these crosses. Although intersectional hybrids have been produced, the easiest and most promising hybrids are within the section Leuce (Einspahr and Winton 1976).

Natural hybrids of Populus tremuloides x P. grandidentata Michx. are relatively common in areas of sympatry (Barnes 1969; Einspahr and Joranson 1960). Einspahr and Joranson suggested phenological differences between the two species prevented large-scale hybridization. Natural hybrids of Populus tremuloides and the exotic P. alba L. have been reported (Heimburger 1936; Peto 1938).

Natural Variation in Trembling Aspen

If acceptable breeding results are to be achieved and the understanding of evolutionary events are to be enhanced, an estimate of the amount and organization of genetic variation in the species is necessary. Ecological and economic interest in trembling aspen has stimulated numerous investigations of its natural variation.

Photoperiodic ecotypes of trembling aspen have been reported by Vaartaja (1960). Intraclonal variation was reported in stem volume, wood specific gravity, and fibre length (van Buijtenen et al. 1959), and leaf morphology (Barnes 1969). Barnes (1969) also reported interclonal variation in phenology, leaf flushing, fall coloration, time of leaf fall, bark color and texture, stem form, and leaf morphology. Einspahr and Benson (1966) reported clinal variation in wood specific gravity in eastern aspen populations. The majority of the variation in wood specific gravity was among populations. Significant variation in wood fibre length was found among populations, stands, and clones. Covington (1975) reported altitudinal variation in bark chlorophyll concentration and reflectance. An inverse relationship existed between either chlorophyll concentration or bark reflectance and elevation. Barnes (1975) examined phenotypic variation of bud, leaf, and twig characters in aspen populations in western North America. South to north clinal variation existed in leaf shape, leaf size, and number of serrations. The majority of the variation was among populations. Barnes suggested a genetic basis for much of the described phenotypic variation. Significant among-family variation for nine of 12 leaf characters was reported by Farmer and Barnes (1978). Intra-family variation was less than inter-family variation, although individuals within a family were not highly uniform.

Electrophoretic analyses of trembling aspen populations in Colorado revealed no significant differences in allelic frequencies of three enzyme loci with changes in elevation (Mitton and Grant 1980). Cheliak (1980) found significant among-population variation in leaf morphology in Alberta trembling aspen populations. A south to north trend existed for smaller, more serrated leaves with increasing leaf base and vein angle acuteness. Electrophoretic analysis indicated high levels of heterozygosity (54%) and polymorphic loci (84%).

The high incidence of Hypoxylon canker in trembling aspen in the Great Lakes region has stimulated numerous investigations of natural variation in resistance to Hypoxylon. Copony and Barnes (1974) examined the incidence of Hypoxylon canker in five Michigan aspen populations. Inter-clonal variation in canker incidence was found to be significant on four sites. Intra-clonal and among population variation were not significant. Inter-clonal variation in canker enlargement was reported by French and Manion (1975). French and Hart (1978) found Hypoxylon cankers to be larger and more frequent in northern Michigan than southern Michigan. They also reported significant interclonal variation in canker development.

Inter-clonal variation in trembling aspen also has been reported for spring frost damage (Egeberg 1963), susceptibility to sulphur dioxide and ozone (Karnosky 1977), decay (Wall 1971), and suckering ability (Schier 1974).

Barnes (1967) introduced evidence supporting large genetic differences between eastern and western aspen populations. This was substantiated by a controlled cross using a Michigan female and an Idaho male, which produced genotypes with highly variable pollen (Barnes 1978).

These morphological and biochemical surveys of natural variation in trembling aspen indicate that the species is extremely variable and that the majority of the variation in many characters is inter-clonal.

C. Study Objectives

Since biochemical analyses of surface waxes of many plant species are lacking (Tulloch 1976), and relatively little is known regarding intraspecific variation in leaf epicuticular wax chemical composition and morphology, a comprehensive investigation of the epicuticular waxes of trembling aspen (Populus tremuloides Michx.) would be beneficial. Such an investigation would contribute to our knowledge of plant waxes in general and would further our understanding of the ecology and genetics of trembling aspen. The study might also provide useful information pertaining to the chemotaxonomic utility of the leaf wax hydrocarbons within the Salicaceae, and to sexual spatial segregation in dioecious plants. The specific objectives of this study were:

1. To determine the composition and temporal variation of the alkane hydrocarbon fraction of leaf epicuticular waxes of trembling aspen.

2. To describe natural variation of aspen leaf wax hydrocarbons among crown positions, clones, and populations; and between shoot types and sexes.
3. To describe natural variation in leaf wax morphology between staminate and pistillate aspen foliage.

A secondary objective was to utilize various multivariate statistical techniques to obtain an appreciation of their value in analyzing biological data.

D. Problem Statement

The specific questions this study addressed were:

1. Do leaf wax alkane concentrations differ throughout the growing season?
2. Are there significant differences in alkane concentrations in leaves from:
    a. different clones,
    b. different crown positions (lower, middle, upper),
    c. the four cardinal directions within each crown position in a clone,
    d. long and short shoots of the same clone?
3. Is there a relationship between leaf wax alkane concentrations and gender in trembling aspen?
4. Do relationships exist between leaf wax alkanes of aspen leaves on various sites and:
    a. latitude,
    b. longitude,

c. elevation,

d. average daily temperature during the growing season (May-September),

e. average daily temperature range during the growing season (May-September), and

f. total growing season (May-September) precipitation?

II. MATERIALS AND METHODS

A. Alkane Analysis

To describe intraspecific variation in the leaf epicuticular wax alkanes of trembling aspen, the study was conducted as four separate experiments. These experiments described temporal, inter- and intraclonal, sexual, and geographic variability in the leaf wax alkanes of trembling aspen. Experiments I, II, and III were conducted on natural aspen clones growing on the Woodbend (Plant Science 160) property of the University of Alberta. Experiment IV was a survey of foliar wax alkanes from clones growing throughout Alberta. Clone identification followed the criteria outlined by Barnes (1969). Since a secondary objective of the study was to obtain an appreciation for multivariate analyses of biological data, brief discussions of the techniques used in the study will be included.

Description of the Woodbend Site

Woodbend is located approximately 17 km southwest of Edmonton and was chosen because of proximity, ease of access, and abundance of naturally occurring aspen clones. The topography of the site is gently undulating to rolling, with sand hills that are remnants of dunes of glacial Lake Edmonton. Poorly drained areas often occur between knolls. Bowser et al. (1963) classified the majority of the soils in the area as belonging to the Culp series of the Podzolic order, although organic soils occur sporadically. Soils of the Culp series are orthic gray wooded soils which have developed on alluvial, aeolian parent material. These soils are characterized by a sandy loam texture, low water storage capacity, less than four percent organic matter in the topsoil, few stones, slightly acidic upper horizons, and are erodable by wind. Trembling aspen is the predominant tree species on the site, although *Betula papyrifera* Marsh., *Pinus banksiana* Lamb., *Picea glauca* (Moench) Voss, and *Populus balsamifera* L. are present.

The average May to September temperature and precipitation in the area is 13°C and 300 mm, respectively. The majority of the precipitation (100 mm) falls in July (Powell and MacIver 1978).

Experiment I - Temporal Variation

Single ramets from four separate clones (two pistillate and two staminate) were randomly selected to examine temporal variation in the leaf wax alkanes. Approximately every two weeks from May 28 to September 21, 1979, approximately 75 leaves of short shoots from the lower, south crown position were collected from each clone. Leaves from each clone were bulked to constitute one sample, resulting in four samples being taken every collection date. All samples were air dried prior to wax extraction.

Surface waxes were extracted by gently agitating the leaves in 100 ml of chloroform for 30 seconds. Solid material was removed from the solution by gravity filtration through glass wool. After evaporation of the cholorform, the white wax residue was dissolved in 25 ml of boiling n-hexane and subjected to column chromatography to separate the hydrocarbon fraction from the polar compounds. The column's 15 x 1 cm stationary phase was composed of 80-200 mesh alumina which had been pretreated by heating at 204°C for 10 hours. The column was packed from an alumina-n-hexane slurry and washed with 50 ml of n-hexane. Delivery of the wax-n-hexane solution to the column was through gravity flow. Hydrocarbons were eluted from the column with 25 ml of n-hexane which, subsequently, was evaporated completely. Sufficient n-hexane was added to the hydrocarbon residue to yield a sample concentration of 2 ug/ul. Throughout the study a new column stationary phase was prepared for every sample, and all bulk n-hexane was distilled to remove impurities. Gas-liquid chromatography (GLC) was used to separate the hydrocarbon homologues.

Two ul of each sample were chromatographed using a Hewlett Packard Model 5830 H gas chromatograph. Injections were made automatically using a Hewlett Packard Model 7672A auto-sampler. Flow rate of the helium carrier gas was 55 ml/min. The chromatograph was fitted with a 2.7 mm by 3.2 mm stainless steel column containing 3 percent SE30 on a 60/80 mesh chromasorb Q non-acid-washed support. Oven and injector temperatures were 230°C and 325°C, respectively. The hydrogen flame ionization detector was maintained at a temperature of 330°C. Identification of hydrocarbon homologues was made by comparison of peak retention times to retention times of known analytical standards and confirmed by mass spectrometry.

The 5830A option method NORM was used to monitor the interrelations of the homologous series. This method uses equation (1) to determine the percent of each homologue present within the sample.

$$I = \frac{(\text{Area } i)\ (\text{Response } i)}{\sum_{n=7} (\text{Area } i \times \text{Response } i)} \times 100 \quad , \quad (1)$$

where: I = concentration of the compound "i"
Area i = area under peak "i"
Response i = compound "i" amount/area from calibration mixture
n = number of peaks

The method NORM provides the concentration of each compound in relative percentage and corrects for variation in detector response to different compounds. These relative percentages were used in all statistical analyses throughout the study.

Significance of differences in alkane concentrations among the nine collection dates were determined using one-way analyses of variance. Percentages were transformed by the inverse sine transformation to minimize non-normality, non-additivity, and heterogeneity of variances in the data. A Student-Newman-Keul's (SNK) multiple comparison test was used to determine those collection dates which exhibited significantly different alkane concentrations (Steele and Torrie 1960). The

Statistical Package for the Social Sciences (SPSS) (Nie _et al_. 1975) computor package was used to compute the analyses. Tests of statistical significance were made at the 5 percent probability level and unless otherwise stated, the 5 percent probability level for testing levels of significance will be standard throughout the study.

Experiment II - Inter-and Intraclonal Variation

To determine if inter- and intraclonal variation existed among the leaf wax alkanes of trembling aspen, four ramets from each of three clones were randomly selected for detailed examination. These clones were unique to this experiment. Crowns of each ramet were subjectively stratified into three sections: lower, middle, and upper. In each stratum approximately 75 leaves were collected from both long and short shoots of branches oriented along cardinal directions. The leaves from each sampling position were bulked to constitute one sample. This sampling scheme (3 clones, 4 ramets per clone, 3 strata per ramet, 4 directions per strata, and 2 shoot types per direction) resulted in 288 samples being collected. Leaves were collected during the last week of August to reduce possible error resulting from temporal variation. The leaves were allowed to air dry prior to wax extraction, column chromatography, and GLC, which followed the conditions previously described.

Percentage data were transformed using the inverse sine transformation. Analyses of variance were computed to determine if significant differences existed among means of clones, strata, direction, and shoot type main effects, and first and second order interactions. Higher order interactions were pooled into the error term. Analyses of variance were computed using AOV 5 (Smillie 1969). Student-Newman Keul's multiple comparison test was used to compare means when significant differences existed.

The clonal growth habit of trembling aspen provides a natural clonal test for estimating genetic and environmental variance components (Barnes 1969). Thus, heritability in the broad sense (σ^2G/σ^2P) may be determined to estimate the extent of the phenotypic variance of a character that is attributable to genetic differences among clones. Broad-sense heritabilities have been estimated for height growth, diameter, branch angle, wood properties, and leaf morphological characters in trembling aspen (Barnes 1969; van Buijtenen *et al*. 1959). To estimate the portion of the phenotypic variation in the leaf wax alkanes that is due to genetic differences among clones, broad-sense heritabilities were calculated from variance components computed from the analyses of variance.

Experiment III - Sexual Variation

Approximately 75 short-shoot leaves were collected from a standardized crown position in three ramets from 18 staminate and 17 pistillate clones from Woodbend to determine if significant differences existed between pistillate and staminate foliar wax alkanes. These clones were identified and permanently marked in the spring of 1979 and were unique to this experiment. Floral morphology was the criteria used to determine clone sex. Foliage was collected in late August of the same year. Surface wax extraction, column chromatography, and GLC conditions were similar to those previously described.

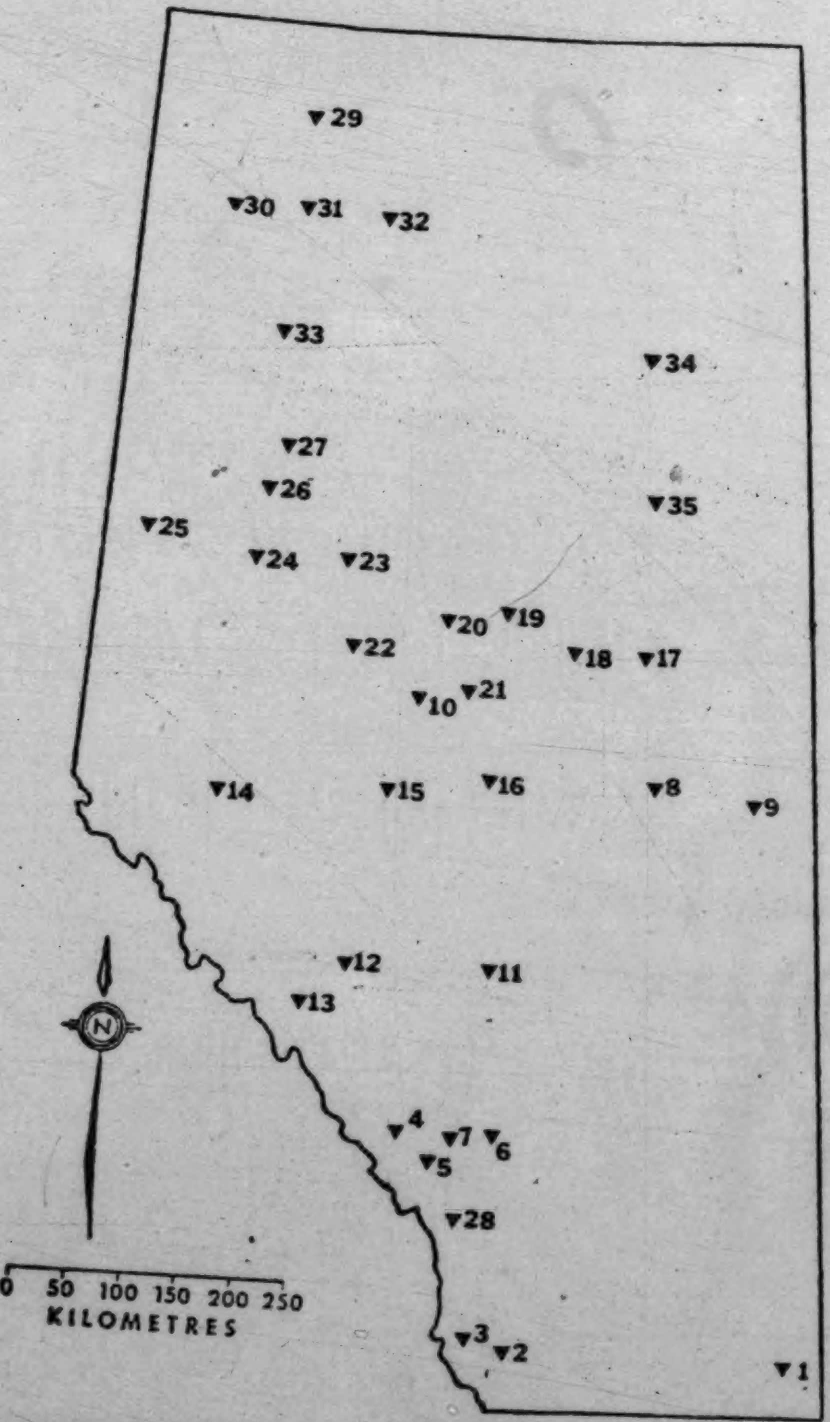
A univariate t-test was used to determine if significant differences existed between the group means for each alkane. Discriminant analysis, using the SPSS routine, was used to compute a discriminant function that statistically separates the two groups (Nie et al. 1975). Input variables were the alkane variables that proved significantly different across the two groups. Discriminant analysis forms one or more linear combinations of the variables in such a way that among-group variation is maximized relative to within-group variation (Green 1978). The maximum number of linear combinations, or discriminant functions, computed is either one less than the number of groups or is equal to the number of discriminating variables if more groups than variables are present. Discriminant functions may be used analytically to elucidate spatial relationships among the groups and to indicate which variables contribute most to group discrimination. They also may be used to classify new cases whose group membership is unknown (Green 1978; Nie et al. 1975).

Hotelling's T-test was used to test whether the two group centroids of the alkane variables included in the discriminant analysis were significantly different. This test is particularly useful if multicollinearity exists among the variables and if hypothesis testing by the univariate t-test is biased by mutual relationships among the predictor variables (Green 1978).

Experiment IV - Geographic Variation

Geographic variation in aspen leaf wax alkanes was elucidated by sampling 35 natural populations throughout Alberta (Fig.3). Sampling was conducted along latitudinal and longitudinal gradients. Descriptions of population locations and three population geographic and environmental parameters are included in Table 1. Leaves of five short shoots from a standardized crown position of a single ramet were collected from five clones per population.

This phase of the study was initiated in conjuction with a related trembling aspen variation study. Single ramets of five pistillate clones from 28 populations initially were identified and permanently marked; open-pollinated seeds were collected from each clone. In late summer of the same growing season, mature leaves were collected from the marked ramets of all populations. Those ramets that could not be relocated or were destroyed were substituted with randomly selected clones from the same population where the missing ramet existed. In addition,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KILOMETRES
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2
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Figure 3 Location of Alberta populations sampled to describe geographic variation in trembling aspen leaf wax alkane hydrocarbons. See Table 1 for site descriptions.

Table 1. Geographic and environmental data for the 35 trembling aspen populations sampled to describe phenotypic variation in the leaf epicuticular wax alkanes.

POPULATION			LAT.	LONG.	ELEV. (M)	AV DAILY TEMP* (C°)	AV. DAILY TEMP. RANGE* (C°)	TOTAL PRECIP.* (MM)
NUMBER	SYMBOL	LOCATION						
1	CHL	CYPRESS HILLS	49.67	110.25	1280	13.0	12.7	280
2	LUN	LUNDBRECK	49.58	114.17	1220	12.0	14.8	320
3	CLM	COLEMAN	49.63	114.57	1370	11.1	16.1	260
4	HHT	HARVEY HEIGHTS	51.13	115.37	1370	11.4	14.6	265
5	KAN	KANANASKIS	50.98	115.08	1520	10.4	17.7	380
6	CGY	CALGARY	51.08	114.27	1160	12.8	12.6	300
7	MOR	MORLEY	51.10	114.60	1220	12.8	12.6	330
8	MUN	MUNDARE	53.35	112.38	680	13.7	13.6	300
9	VER	VERMILION	53.33	110.83	610	13.1	14.1	280
10	BLU	BLUE RIDGE	54.22	115.33	730	11.8	14.6	340
11	SYL	SYLVAN LAKE	52.32	114.58	980	12.2	13.6	370
12	NOR	NORDEGG	52.48	116.10	1370	9.5	17.2	390
13	CLI	MT. CLINE	52.02	116.60	1400	9.5	17.2	390
14	BRU	BRULE LAKE	53.38	117.72	980	11.5	16.0	330
15	EDS	EDSON	53.60	116.38	910	11.7	15.1	370
16	WAB	WABUMUN	53.58	114.58	730	12.3	15.2	335
17	LLB	LAC LA BICHE	54.67	111.93	550	12.5	13.5	300
18	ATH	ATHABASCA	54.78	113.32	600	13.0	16.8	295
19	SLK	SLAVE LAKE	55.37	114.75	580	12.2	13.4	300
20	SWH	SWAN HILLS	55.13	115.32	640	12.4	13.8	265
21	FTA	FT. ASSINIBOINE	54.37	114.88	670	12.6	14.7	300
22	FOX	FOX CREEK	54.32	116.57	850	11.0	11.1	420
23	VVW	VALLEYVIEW	55.07	117.30	670	13.1	14.0	270

continued

Table 1. cont'd

24	GPR	GRANDE PRAIRIE	55.05	118.08	670	12.9	13.0	250
25	HYT	HYTHE	55.42	119.43	790	12.5	12.8	265
26	RYC	RYCROFT	55.72	118.73	620	12.7	14.2	225
27	PEA	PEACE RIVER	56.22	117.13	550	12.5	13.9	205
28	HWD	HIGHWOOD PASS	50.66	114.85	1830	8.9	10.0	290
29	MEA	MEANDER R.	59.03	117.65	320	11.2	9.8	330
30	CHI	CHINCHAGA R.	58.60	118.32	365	11.4	10.8	330
31	HLL	HIGH LEVEL	58.52	117.10	320	12.1	15.4	330
32	FTV	FT. VERMILION	58.40	115.98	275	12.8	13.6	210
33	TWL	TWIN LAKES	57.40	117.53	720	12.9	14.6	250
34	FTM	FT. MACKAY	57.18	111.62	240	12.5	14.0	290
35	HSR	HOUSE RIVER	55.63	112.13	640	12.1	14.1	412

* Environmental information was obtained from Environment Canada's Climatological Station data; values are based on data collected from May through September.

leaves from five randomly selected clones from seven northerly populations were collected. Gender of these randomly selected clones was unknown. Wax extraction and analysis was conducted under the previously described conditions.

Descriptive statistics, using the CONDESCRIPTIVE routine of SPSS, were computed for each alkane from all populations. One-way analyses of variance and SNK multiple comparison tests were performed on the inverse sine transformed data to determine if population alkane means were significantly different. Alkane means for each population were used in Pearson product moment correlation and cluster analyses.

Pearson product moment correlation was used to assess the strength of the linear relationship among alkanes and between the alkanes and the following geographical and environmental parameters: latitude, longitude, and elevation; and average growing season daily temperature, average daily temperature range, and total precipitation. All of the environmental data represent the period of May through September and were obtained from Environment Canada's Climatological Station Data (Anonymous 1975). The data used for each population were from the meteorological station nearest the collection site and were based on thirty year averages.

Two multivariate statistical techniques, cluster analysis and multiple discriminant analysis, were used to describe spatial relationships among the aspen populations. Cluster analysis seeks to produce K groups derived from P variables for each of N objects in such a way that K is much less than N. Objects within a group are similar with regard to the P variables, while objects in different groups are dissimilar. Cluster analysis was performed using Clustan (Wishart 1978). Initially, a distance matrix based on squared euclidian distance was calculated. Ward's method of hierarchical fusion was used to group the populations. This clustering algorithm seeks to minimize the within-group sum of squares, which is be defined as the sum of the distances from each object to the center of the cluster to which that object belongs. The minimum increase in total within-group sum of squares criterion was used to select the optimum number of groups. Principal components also were computed by Clustan.

Multiple discriminant analysis was performed using the alkanes which proved significantly different among the populations in the one-way analyses of variance as input variables. The direct method of variable input was used (Nie _et_ _al_. 1975). Population centroids were plotted along the first two discriminant axes to reveal spatial relationships in reduced space.

The groups delineated by cluster and discriminant analysis were characterized by one-way analysis of variance and discriminant analysis using the original six input variables. Grouping of populations was based on the results of the cluster analysis.

B. Scanning Electron Microscopy

Single leaves were collected from the upper and lower crown positions of single ramets from staminate and pistillate clones for observation by scanning electron microscopy. The fresh samples were fixed to aluminum mounting discs with silver paste and sputter coated with gold to a thickness of approximately 400A. An International Scientific Instruments (model ISI-60) scanning electron microscope was used to make the observations. Operating conditions of the microprobe were 15KV and 80-100 microamps.

III. RESULTS

A. Alkane Analysis

Gas-liquid chromatography of the hydrocarbon fraction of trembling aspen leaf wax revealed the presence of seven major compounds. Mass spectrometry and comparison of peak retention times with those of analytical standards indicated all of the compounds were homologues of unbranched, saturated alkane hydrocarbons. Carbon numbers of the homologues ranged from 22 to 29. A single branched 28 carbon iso-alkane (iso-octacosane) occurred sporadically throughout the sample analysis; however, due to the infrequent nature of the compound, it was excluded from statistical analyses. Since all homologues analyzed were unbranched, saturated normal-alkanes, they will be referred to as nC-N throughout this thesis, where "N" represents the number of carbon atoms present in the n-alkane.

A profile approximating the concentrations of aspen leaf wax alkane hydrocarbons indicates odd-carbon-numbered alkanes predominate qualitatively and quantitatively (Fig. 4). Heptacosane (nC27) comprised approximately 55 percent of the alkane fraction, while the three even-carbon-numbered alkanes, docosane (nC22), tetracosane (nC24), and hexacosane (nC26), together constituted approximately 10 percent of the average sample.

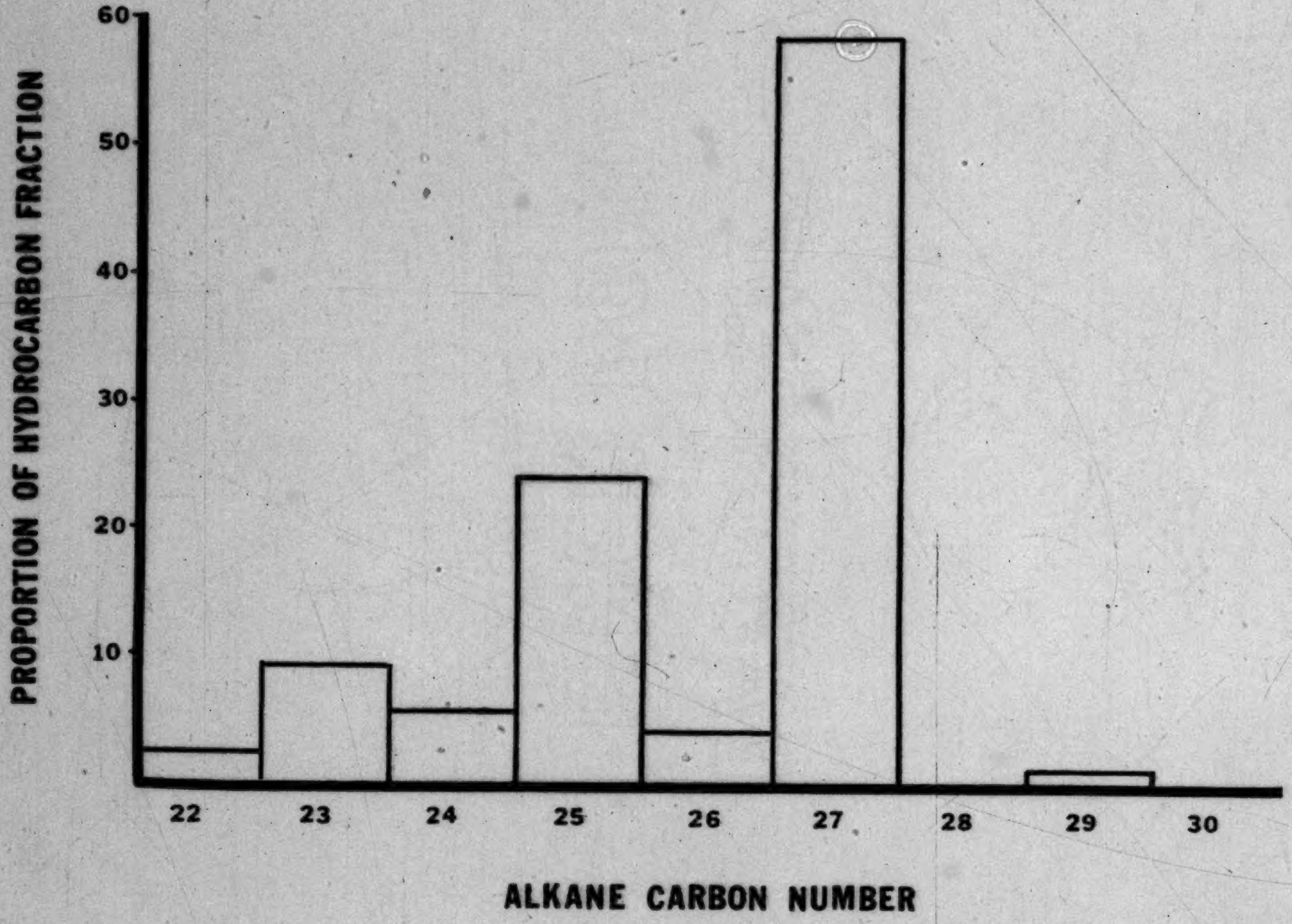

Figure 4 Distribution of n-alkanes comprising the alkane hydrocarbon fraction of Populus tremuloides Michx. foliar epicuticular wax.

Temporal Variation

Quantities of all of the alkanes except tricosane (nC23) and pentacosane (nC25) varied significantly ($p<.05$) over the growing season (Table 2). Within those alkanes whose collection dates were significantly different, the major change in relative composition occurred in mid-June, approximately six weeks following leaf flush. Collections following this transition period revealed a marked, but insignificant, increase in nC23 and nC25, and decrease in nC27. The wax deposited initially was composed primarily of compounds nC25 and nC27, while the even-carbon-numbered compounds appeared approximately one month following leaf flush. Compound nC27 predominanted throughout the season.

Table 2. Percentage composition of the alkane hydrocarbon fraction of trembling aspen leaf surface wax at nine intervals throughout the season. # *

COLLECTION DATE	ALKANE CARBON NUMBER						
	22*	23	24*	25	26*	27*	29*
MAY 28	0.00^{1}	0.80	0.00^{1}	24.28	0.00^{1}	68.47^{1}	0.00^{1}
JUNE 12	0.00^{1}	5.40	0.46^{2}	22.99	0.33^{2}	68.94^{1}	0.80^{2}
JUNE 27	1.49^{2}	4.74	4.02^{3}	20.92	2.83^{3}	$64.95^{1\,2}$	1.43^{2}
JULY 9	2.07^{2}	4.77	5.35^{3}	20.70	2.94^{3}	$62.74^{1\,2}$	1.23^{2}
JULY 20	0.32^{1}	5.23	5.07^{3}	22.00	2.52^{3}	$64.45^{1\,2}$	0.25^{1}
AUG 7	2.19^{2}	7.16	5.72^{3}	23.96	2.50^{3}	$57.51^{1\,2}$	0.90^{2}
AUG 20	1.77^{2}	7.41	5.87^{3}	24.86	2.78^{3}	$56.16^{1\,2}$	0.86^{2}
SEPT 4	1.80^{2}	8.57	6.35^{3}	25.65	2.96^{3}	$53.84^{\ 2}$	0.85^{2}
SEPT 17	1.65^{2}	9.16	6.67^{3}	25.77	2.93^{3}	$52.76^{\ 2}$	0.80^{2}

# Values are means based on 4 clones.

* ANOVA F-ratios were significantly different at $p<.05$; where ANOVA F-ratios were significantly different, values superscripted by the same number are not significantly different at $=.05$.

Inter-and Intraclonal Variation

Considerable variability existed among the means of the leaf wax alkanes from the various sampling positions (Table 3). Of the four main effects, direction within crown had the least effect on alkane concentration (Table 4). Significant differences among clone means were found for all of the alkanes. Multiple comparison tests indicated the means of all three clones were significantly different for all of the alkanes except nC25 and nC27, where two of the clones were not significantly different (Table 3). Differences among strata means were significant for all of the alkanes except nC25. Two major relationships existed between leaf wax alkane concentration and crown position. Concentrations of all of the alkanes, except nC27 and nC29, generally increased with increasing crown position. Alkanes nC27 and nC29 generally decreased with increasing crown position. Alkane nC23 was the only compound exhibiting a significant difference among the four cardinal directions. Significant differences were found between long-shoot and short-shoot leaves for all of the alkanes except nC25 and nC29.

Among the significant first-order interactions, the clone X strata interaction in nC27, and the clone X strata and clone X direction interactions in nC24 may be of interest since they were significant and their variance ratios were relatively large. Since the scope of this study deals primarily with the main effects, further interpretation of these interactions was not carried out. No

Table 3. Mean percentages of the epicuticular wax alkanes of trembling aspen foliage sampled to describe inter- and intraclonal variability.+ * #

SAMPLE	ALKANE CARBON NUMBER						
POSITION	22	23	24	25	26	27	29
CLONE (n=96)							
one	2.62^{1}	6.00^{1}	7.87^{1}	22.11^{1}	3.67^{1}	56.56^{1}	0.01^{1}
two	0.01^{2}	8.57^{2}	4.68^{2}	30.45^{2}	2.41^{2}	56.68^{1}	0.01^{2}
three	1.03^{3}	5.64^{3}	5.20^{3}	22.45^{1}	2.04^{3}	63.04^{2}	0.00^{3}
STRATA (n=96)							
low	0.01^{1}	5.60^{1}	5.38^{1}	22.39	2.42^{1}	61.77^{1}	0.01^{1}
middle	1.31^{2}	6.58^{2}	5.82^{2}	24.67	2.67^{2}	59.00^{2}	0.01^{2}
top	2.18^{3}	7.93^{3}	6.35^{3}	27.77	2.92^{3}	55.54^{3}	0.00^{3}
DIRECTION (n=72)							
south	1.12	6.46^{1}	5.75	23.32	2.70	59.30	0.01
east	1.23	6.67^{2}	5.84	23.53	2.68	58.68	0.01
north	1.46	6.86^{2}	5.99	29.30	2.66	58.39	0.01
west	1.17	6.71^{2}	5.78	23.64	2.62	58.77	0.01
SHOOT TYPE (n=144)							
short	0.01^{1}	6.45^{1}	5.42^{1}	26.59	2.57^{1}	59.59^{1}	0.01
long	1.56^{2}	6.91^{2}	6.27^{2}	23.27	2.76^{2}	57.96^{2}	0.01

+ Four ramets of three clones were sampled; from each ramet long and short-shoot leaves from cardinal directions in each of three crown strata were analyzed.

* F-ratios for superscripted groups are significant ($p<.05$); within each group, means superscripted with the same number are not significantly different ($p<.05$).

# "n" is the number of observations per sampling position.

Table 4. Summary of ANOVA F-values for seven epicuticular wax alkanes of trembling aspen foliage from the Woodbend site.

SOURCE OF VARIATION	df	ALKANE CARBON NUMBER						
		22	23	24	25	26	27	29
CLONE (A)	2	172.02*	351.49*	236.75*	3.43*	488.35*	150.52*	223.34*
STRATA (B)	2	114.93*	158.15*	35.28*	1.16	46.69*	102.14*	33.91*
DIRECTION (C)	3	2.32	3.44*	1.10	0.98	.79	2.09	1.19
SHOOT TYPE (D)	1	64.91*	70.25*	174.06*	1.32	30.15*	79.70*	3.13
INTERACTIONS								
A X B	4	1.21	2.86*	7.28*	1.03	3.05*	9.79*	2.89
A X C	6	2.07	0.68	5.05*	0.91	0.83	1.46	0.70
A X D	2	1.14	1.50	4.75*	1.07	3.45*	3.07*	0.52
B X C	6	2.27*	2.78*	2.37*	1.03	0.83	2.80*	0.76
B X D	2	0.11	0.52	0.07	0.97	2.27	0.56	0.13
C X D	3	1.38	0.31	1.58	1.01	0.08	0.87	0.82
Error MS	228	3.50	0.74	0.53	261.82	0.37	1.39	0.67

* F is significant at p<.05 level.

second-order interactions were significant.

Broad sense heritability estimates were high for all of the alkanes except nC25, which was virtually zero (Table 5). Heritabilities in the broad sense for the other six alkanes ranged from .61 to .84, indicating the majority of the phenotypic variation in these characters is attributable to genetic differences among clones.

Table 5. Components of variance and broad-sense heritability estimates for seven trembling aspen leaf wax alkanes. #

COMPONENTS	ALKANE CARBON NUMBER						
	22	23	24	25	26	27	29
AMONG CLONES	7.11	3.19	4.03	6.66	2.10	4.70	1.83
WITHIN CLONES	3.99	0.87	1.43	263.46	0.41	3.01	0.79
HERITABILITY (BS)	.64	.78	.74	.02	.84	.61	.70

# Based on 4 ramets from 3 clones from the Woodbend site.

Sexual Variation

Significant differences existed between the means of staminate and pistillate foliar wax alkanes in four of the seven compounds (Table 6). Coefficients of variation were similar in both groups.

The single extractable discriminant function was highly significant (p<.001). The discriminant function, which was derived using the four significantly different alkanes as

Table 6. Mean percentages, standard deviations, and coefficients of variation of staminate and pistillate aspen foliar wax alkanes.# +

ALKANE CARBON NUMBER	STAMINATE (N=18)			PISTILLATE (N=17)		
	MEAN	SD	COEFF VAR	MEAN	SD	COEFF VAR
22	1.51	0.78	52	1.37	1.02	74
23*	10.90	3.37	31	7.40	1.92	26
24	6.09	1.13	19	6.25	1.39	22
25*	24.43	2.02	8	22.30	3.09	14
26*	2.74	0.56	20	3.46	0.72	21
27*	53.65	5.12	10	58.43	5.37	9
29	0.67	0.30	45	0.78	0.23	30

# Alkanes are from short-shoot foliage from aspen clones on the Woodbend site.

+ The value for each clone (N) was a mean based on 3 observations (ramets) per clone.

* Means are significantly different at $p<.05$.

input variables, provided moderate group discrimination (Wilk's Lamba = .60) and correctly classified 83 percent of the aspen clones to the appropriate sex. A plot of discriminant scores of individual group members and group centroids indicated pistillate clones were characterized by negative factor scores while staminate clones were characterized by positive factor scores (Fig. 5). Highest positive loading on the discriminant function is on nC23 (0.97), while the highest negative loading is on alkane nC26 (-0.44) (Table 7). The F statistic calculated by Hotelling's T-test was 4.83, indicating the two group centroids were significantly different {F (α=.05)=2.92}.

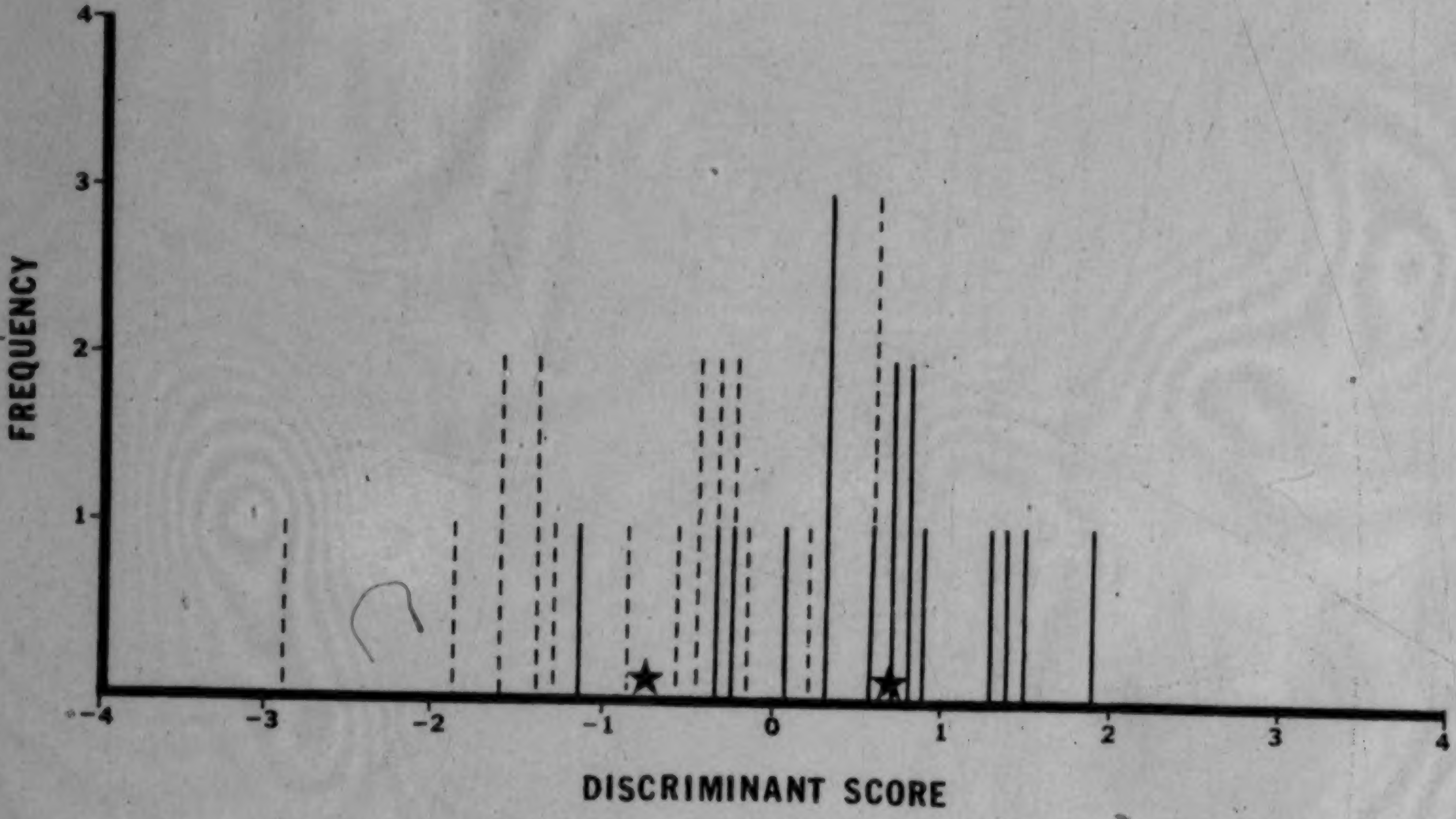

Figure 5. Plot of discriminant scores of staminate and pistillate trembling aspen clones using n-alkanes nC23, nC25, nC26, and nC27 as input variables. Staminate and pistillate clones are represented by —— and - - - - , respectively. ★ represent the group centroids.

Table 7. Standardized canonical discriminant function (CDF) coefficients derived for four discriminating variables used in classifying 18 staminate and 17 pistillate trembling aspen clones from the Woodbend site.

ALKANE CARBON NUMBER	CDF COEFFICIENT
23	0.97099
25	0.27894
26	-0.43868
27	0.43117

Geographic Variation

A summary of the descriptive statistics for the seven leaf wax alkanes from the 35 populations indicated that alkanes nC25 and nC27 are the least variable, while nC22 and nC29 are the most variable (Tables 8-14). The F-values comparing the means of the Alberta populations were significant for all of the alkanes except nC29 (Table 15).

Pearson product moment correlations between the alkane, geographic, and environmental variables revealed several strong and significant correlations among the variables (Table 16). Alkane nC27 was significantly correlated with all of the alkanes except nC29. A strong inverse relationship (r=-.92) existed between nC27 and nC23, while moderately strong inverse relationships existed between nC27 and both nC24 and nC25 (r=-.65 and -.69). Alkane nC29 exhibited a weak, though significant, relationship with nC26.

Among the geographic variables, elevation exhibited the

Table 8. Descriptive statistics of leaf surface wax hydrocarbon nC22 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	.37	.52	.23	140.50	1.06
LUN	.72	.66	.30	91.60	1.31
CLM	.93	.52	.23	55.90	1.23
HHT	.99	.91	.40	91.90	1.78
KAN	1.13	.73	.34	64.60	1.85
CGY	.47	.65	.29	138.20	1.28
MOR	.25	.57	.25	228.00	1.27
MUN	.88	.58	.26	65.90	1.45
VER	.82	.76	.34	92.60	1.54
BLU	1.20	1.17	.52	97.50	2.65
SYL	1.87	.42	.19	22.40	.91
NOR	.00	.00	.00	.00	.00
CLI	1.49	1.38	.62	92.60	3.62
BRU	1.85	1.08	.48	58.30	2.64
EDS	2.86	.63	.28	22.00	1.68
WAB	2.50	.73	.33	29.20	1.86
LLB	1.55	.42	.19	27.00	1.10
ATH	1.38	.94	.42	68.10	2.44
SLK	1.21	.73	.33	60.30	1.86
SWH	1.19	1.11	.50	93.20	2.33
FTA	1.28	.76	.34	59.30	1.83
FOX	1.34	.78	.35	58.20	2.04
VVW	1.29	.47	.21	36.40	1.24
GPR	1.16	.90	.40	77.50	2.39
HYT	1.32	.41	.18	31.00	.96
RYC	.99	.69	.31	69.60	1.78
PEA	1.25	.61	.27	48.80	1.37
HWD	1.62	.26	.11	16.00	.60
MEA	.00	.00	.00	.00	.00
CHI	.97	.55	.25	56.70	1.37
HLL	1.30	.31	.14	23.80	.61
FTV	1.69	.40	.18	23.60	.95
TWL	.24	.54	.24	225.00	1.21
FTM	2.10	.74	.33	35.00	1.90
HSR	1.31	.75	.34	57.20	1.91

* Based on 5 clones per population.
+ Expressed as % of total sample

Table 9. Descriptive statistics of leaf surface wax hydrocarbon nC23 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	5.45	1.02	.46	18.70	2.59
LUN	6.03	3.02	1.35	50.00	7.55
CLM	5.98	1.70	.76	28.40	4.43
HHT	6.11	1.90	.85	31.00	4.98
KAN	1.13	.73	.34	64.60	1.85
CGY	5.01	1.19	.53	23.70	3.09
MOR	6.07	.62	.28	10.20	1.57
MUN	12.26	1.96	.88	15.90	4.23
VER	11.57	4.22	1.89	36.40	10.01
BLU	8.65	.99	.44	11.40	2.38
SYL	6.77	1.18	.53	17.40	2.80
NOR	4.22	.52	.23	12.30	1.41
CLI	4.19	1.56	.70	37.20	3.92
BRU	6.60	2.31	1.03	35.00	6.09
EDS	10.11	2.32	1.04	22.90	4.64
WAB	8.76	1.92	.86	21.90	5.18
LLB	10.30	3.07	1.37	29.80	6.90
ATH	10.44	4.40	1.97	42.10	11.60
SLK	8.85	2.74	1.22	30.90	6.48
SWH	8.37	3.31	1.48	39.50	8.55
FTA	7.50	5.02	2.24	66.90	11.71
FOX	9.49	2.01	.90	21.11	4.83
VVW	7.38	1.81	.81	24.50	4.64
GPR	10.90	4.19	1.87	38.40	11.08
HYT	7.33	1.24	.55	16.90	3.00
RYC	6.55	.92	.41	14.00	2.41
PEA	8.32	3.25	1.45	39.00	8.08
HWD	4.71	.72	.32	15.20	1.57
MEA	5.20	1.41	.63	27.10	3.12
CHI	5.21	1.29	.58	24.70	3.18
HLL	6.22	2.41	1.08	38.70	6.08
FTV	6.44	1.00	.44	15.50	2.35
TWL	5.27	1.14	.51	21.60	2.63
FTM	9.32	3.60	1.61	38.60	8.58
HSR	6.05	1.44	.64	23.80	3.46

* Based on 5 clones per population.
+ Expressed as % of total sample

Table 10. Descriptive statistics of leaf surface wax hydrocarbon nC24 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	3.45	.69	.31	20.00	1.78
LUN	3.58	.99	.44	27.60	2.81
CLM	4.13	.93	.41	22.50	2.46
HHT	4.24	1.27	.57	29.90	3.12
KAN	4.64	.93	.42	20.00	2.42
CGY	4.00	.86	.39	21.50	2.28
MOR	4.19	.76	.34	18.10	1.53
MUN	6.22	1.12	.50	18.00	2.66
VER	5.70	.90	.40	15.70	2.23
BLU	6.00	1.20	.54	20.00	3.00
SYL	4.92	1.20	.31	13.80	1.79
NOR	4.93	1.59	.71	32.20	3.82
CLI	4.76	2.52	1.13	52.90	6.22
BRU	5.36	1.41	.63	26.30	3.43
EDS	6.87	1.32	.59	19.20	3.35
WAB	5.89	.76	.34	12.90	1.96
LLB	6.55	1.68	.75	25.60	3.79
ATH	4.82	.70	.31	14.50	1.89
SLK	5.57	.99	.44	17.70	2.06
SWH	4.28	1.66	.74	38.70	4.26
FTA	4.33	1.38	.62	31.88	3.82
FOX	5.32	.65	.29	12.22	1.49
VVW	4.36	1.50	.67	34.40	3.94
GPR	4.53	1.47	.66	32.40	3.72
HYT	3.98	.66	.30	16.50	1.55
RYC	3.27	.63	.28	19.20	1.49
PEA	4.80	1.00	.44	20.80	2.72
HWD	4.07	.42	.19	10.30	.93
MEA	3.77	.50	.22	13.20	1.26
CHI	4.44	.66	.29	14.80	1.73
HLL	4.29	.58	.26	13.50	1.33
FTV	4.96	.55	.25	11.00	1.38
TWL	3.52	.75	.34	21.00	1.93
FTM	5.28	.67	.30	12.60	1.66
HSR	4.58	.88	.39	19.20	2.35

* Based on 5 clones per population.
+ Expressed as % of total sample

Table 11. Descriptive statistics of leaf surface wax hydrocarbon nC25 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	29.63	2.19	.98	7.30	5.84
LUN	23.14	3.69	1.65	15.90	10.06
CLM	24.93	1.42	.64	5.60	3.32
HHT	23.92	4.34	1.94	18.10	10.71
KAN	25.29	3.84	1.72	15.10	10.05
CGY	24.54	4.43	1.98	18.00	9.96
MOR	27.44	2.69	1.20	9.80	6.29
MUN	30.17	3.10	1.39	10.20	6.96
VER	28.24	2.38	1.06	8.70	4.67
BLU	22.09	4.80	1.82	18.40	9.62
SYL	27.36	2.29	1.02	8.30	5.93
NOR	21.94	3.15	1.41	14.30	6.42
CLI	23.32	5.44	2.44	23.30	14.90
BRU	22.69	4.99	2.23	21.90	13.48
EDS	23.79	2.56	1.15	10.70	6.29
WAB	23.35	1.73	.78	7.40	4.69
LLB	26.90	1.07	.48	3.90	2.71
ATH	25.83	3.90	1.74	15.00	10.61
SLK	28.40	2.02	.90	7.10	5.13
SWH	25.38	3.19	1.42	12.50	7.43
FTA	23.81	3.36	1.50	14.10	8.32
FOX	23.86	6.93	3.10	29.00	16.51
VVW	28.17	4.50	2.01	15.90	12.10
GPR	30.79	3.42	1.53	11.10	8.66
HYT	26.77	5.08	2.27	18.90	12.76
RYC	27.99	2.30	1.03	8.20	6.15
PEA	28.51	1.78	.79	6.20	4.13
HWD	22.29	2.65	1.18	11.80	5.67
MEA	25.29	4.05	1.81	16.00	8.67
CHI	24.23	1.85	.83	7.60	5.20
HLL	24.04	3.73	1.67	15.50	9.95
FTV	29.59	2.58	1.15	8.70	6.60
TWL	23.36	1.91	.86	8.10	3.91
FTM	27.05	2.02	.91	7.40	4.49
HSR	24.69	6.10	2.73	24.70	15.10

* Based on 5 clones per population.
+ Expressed as % of total sample

Table 12. Descriptive statistics of leaf surface wax hydrocarbon nC26 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	1.89	.54	.24	28.50	1.26
LUN	2.09	.62	.28	29.60	1.55
CLM	1.75	.31	.14	17.70	.72
HHT	2.16	.78	.35	36.10	2.03
KAN	2.10	.89	.40	42.30	2.34
CGY	1.41	.33	.15	23.40	.76
MOR	1.78	.44	.20	24.70	.96
MUN	1.41	.21	.10	14.80	.53
VER	1.62	.56	.25	34.50	1.36
BLU	3.62	1.48	.66	40.80	3.46
SYL	1.50	.24	.10	16.00	.61
NOR	3.71	1.59	.71	42.80	3.83
CLI	2.17	.88	.40	40.50	2.17
BRU	2.10	.45	.20	21.40	.92
EDS	2.19	.53	.24	24.20	1.05
WAB	2.93	.52	.23	17.70	1.41
LLB	2.23	.31	.14	13.90	.77
ATH	1.75	.38	.17	21.70	1.05
SLK	2.51	.42	.19	16.70	1.08
SWH	1.90	.31	.14	16.30	.69
FTA	2.33	1.05	.47	45.00	2.44
FOX	3.68	1.96	.88	53.20	4.56
VVW	1.50	.64	.29	42.60	1.39
GPR	1.20	.38	.17	31.60	.95
HYT	2.02	.92	.41	45.50	2.29
RYC	1.23	.32	.14	26.00	.88
PEA	1.45	.42	.19	28.90	1.06
HWD	2.16	.46	.21	21.20	1.07
MEA	2.54	.66	.30	25.90	1.46
CHI	2.63	.62	.28	23.50	1.50
HLL	1.93	.68	.30	35.20	1.64
FTV	2.01	.41	.18	20.30	.89
TWL	2.83	.92	.41	14.40	2.39
FTM	1.63	.26	.12	15.90	.62
HSR	2.75	1.09	.49	39.60	2.59

* Based on 5 clones per population.
+ Expressed as % of total sample

Table 13. Descriptive statistics of leaf surface wax hydrocarbon nC27 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	59.23	2.82	1.26	4.70	7.65
LUN	64.25	7.03	3.14	10.90	17.55
CLM	61.96	3.62	1.62	5.80	9.95
HHT	62.59	7.39	3.30	11.80	19.89
KAN	59.74	4.68	2.10	7.80	12.51
CGY	64.20	5.44	2.43	8.40	13.28
MOR	59.99	3.25	1.45	5.40	8.61
MUN	49.05	3.36	1.50	6.80	8.53
VER	51.94	6.32	2.83	12.10	17.14
BLU	58.44	6.07	2.71	10.30	14.58
SYL	57.58	2.70	1.20	4.60	6.97
NOR	65.19	1.49	.67	2.20	4.01
CLI	64.12	9.14	4.09	14.20	22.10
BRU	61.00	8.67	3.88	14.20	24.29
EDS	54.03	5.72	2.56	10.50	12.01
WAB	56.46	3.72	1.66	6.50	9.07
LLB	52.48	5.76	2.58	10.90	12.53
ATH	55.44	9.13	4.08	16.40	24.97
SLK	53.33	3.71	1.66	6.90	8.69
SWH	58.42	8.59	3.84	14.70	22.00
FTA	60.22	8.97	4.01	14.80	23.03
FOX	56.32	6.46	2.89	10.80	15.03
VVW	56.94	6.16	2.75	10.80	15.03
GPR	51.40	6.16	2.76	11.90	17.25
HYT	58.55	5.55	2.48	9.40	14.28
RYC	59.63	3.04	1.36	5.00	7.54
PEA	55.47	5.14	2.30	9.20	12.13
HWD	65.14	2.60	1.16	3.90	6.18
MEA	63.19	5.40	2.41	8.50	12.02
CHI	62.21	2.65	1.18	4.20	6.46
HLL	62.09	6.29	2.81	10.10	15.89
FTV	55.19	3.07	1.37	5.50	8.07
TWL	64.61	2.28	1.02	3.50	5.50
FTM	54.24	5.03	2.25	9.20	12.53
HSR	60.63	6.20	2.77	10.20	14.85

* Based on 5 clones per population.

+ Expressed as % of total sample

Table 14. Descriptive statistics of leaf surface wax hydrocarbon nC29 from 35 trembling aspen populations in Alberta.

POPULATION	MEAN*+	SD	SE OF MEAN	COEFF VAR	RANGE
CHL	.00	.00	.00	0.00	.00
LUN	.19	.42	.19	221.00	.95
CLM	.32	.51	.23	159.00	1.17
HHT	.00	.00	.00	0.00	.00
KAN	.70	.82	.37	117.80	2.01
CGY	.38	.56	.25	147.30	1.25
MOR	.28	.38	.17	135.70	.73
MUN	.00	.00	.00	0.00	.00
VER	.11	.26	.11	236.30	.57
BLU	.00	.00	.00	0.00	.00
SYL	.00	.00	.00	0.00	.00
NOR	.00	.00	.00	0.00	.00
CLI	.34	.48	.21	141.10	.97
BRU	.40	.59	.26	147.50	1.32
EDS	.14	.32	.14	228.50	.72
WAB	.10	.23	.10	230.00	.52
LLB	.00	.00	.00	0.00	.00
ATH	.34	.46	.21	135.20	.89
SLK	.15	.34	.15	226.60	.75
SWH	.46	.75	.34	163.00	1.72
FTA	.53	.50	.22	94.00	1.07
FOX	.00	.00	.00	0.00	.00
VVW	.36	.52	.23	144.40	1.11
GPR	.00	.00	.00	0.00	.00
HYT	.00	.00	.00	0.00	.00
RYC	.33	.48	.21	145.40	1.04
PEA	.00	.00	.00	0.00	.00
HWD	.00	.00	.00	0.00	.00
MEA	.00	.00	.00	0.00	.00
CHI	.31	.44	.20	141.90	.92
HLL	.13	.29	.13	223.00	.64
FTV	.10	.23	.10	230.00	.51
TWL	.18	.39	.18	216.66	.88
FTM	.38	.53	.24	139.40	1.08
HSR	.00	.00	.00	0.00	.00

* Based on 5 clones per population.
+ Expressed as % of total sample

6

strongest relationship with the alkanes. Three of the alkanes (nC23, nC25, and nC27) showed a significant relationship with elevation. Correlation coefficients between the variables were -.45, -.37, and .50, respectively. No significant correlations were found between the alkane variables and latitude. Two weak, but significant, correlations (r=-.29, -.27) existed between longitude and nC23 and nC24, respectively.

Table 15. Summary of ANOVA-F values for leaf wax alkanes from 35 Alberta trembling aspen populations. +

SOURCE OF VARIATION	df	ALKANE CARBON NUMBER						
		22*	23*	24*	25*	26*	27*	29
AMONG POPULATIONS	34	3.41	4.57	3.17	2.45	3.80	2.96	1.33

+ Alkanes of short-shoot leaves from the lower, south crown position in single ramets of five clones per population were used in the analysis.

* Values are significant at $p<.05$.

Among the environmental variables, average daily temperature throughout the growing season exhibited the strongest correlation with the leaf wax alkanes . Moderately strong relationships were found to exist between average seasonal daily temperature and alkanes nC23, nC25, nC26, and nC27. These relationships were similar to the pattern of alkane variability observed within the crown; that is, nC23 and nC25 concentrations increased with increased crown position, while the inverse was true for nC27. Total

Table 16. Pearson product moment correlation coefficients between alkane, geographic, and environmental variables. +

	nC22	nC23	nC24	nC25	nC26	nC27	nC29
nC22	-	-	-	-	-	-	-
nC23	.39*	-	-	-	-	-	-
nC24	.59*	.67*	-	-	-	-	-
nC25	-.05	.45*	.02	-	-	-	-
nC26	-.08	-.21	.21	-.65*	-	-	-
nC27	-.42*	-.92*	-.65*	-.69*	.31*	-	-
nC29	.02	.14	-.20	-.12	-.25*	.18	-
LATITUDE	.11	.16	.07	.17	.07	-.22	-.08
LONGITUDE	-.01	-.29*	-.27*	-.19	.09	.30*	.05
ELEVATION	-.17	-.45*	-.23	-.37*	.09	.50*	.07
PRECIP. #	.12	-.13	.31*	-.52*	.58*	.21	-.02
AV. DAILY TEMP #	-.02	.53*	.04	.65*	-.49*	-.58*	.01
AV. DAILY TEMP RANGE#	.17	.01	.18	-.23*	.01	.05	.37*

+ Coefficients are based on data collected from 35 Alberta trembling aspen populations; population values were means of 5 observations per population.

# Based on data collected from May through September.

* Significant at $p<.05$.

precipitation throughout the growing period also was moderately correlated with nC24, nC25, and nC26. Two weak, but significant, correlations were found between each of alkanes nC25 and nC27 and average seasonal daily temperature range.

Cluster analysis, using the seven leaf wax alkanes as input variables, reduced the 35 populations to two groups (Fig. 6). The two cluster groups are characterized readily by the geographic location and elevation of their constituent cases. Group one, labelled High Elevation, is composed primarily of high elevation and extreme northern populations. Group two, labelled Central, is composed primarily of low elevation populations across central Alberta. Figure 7 illustrates the cluster groups in relation to the geographic locations of 35 populations.

Principal components analysis performed by Clustan reduced the seven variable alkane data set to three principal components using the minimum eigenvalue one criterion (Isebrands and Crow 1975). These three principal components explained 87 percent of the variance in the data set. The first two components accounted for 70 percent of the variance within the data set. Based on variable loadings, axis one could be labelled nC23 and nC27, while axis two could be labelled nC26. Ordination of the populations along the first two principal components produced results similar to those produced by Ward's method of hierarchical fusion (Fig. 8).

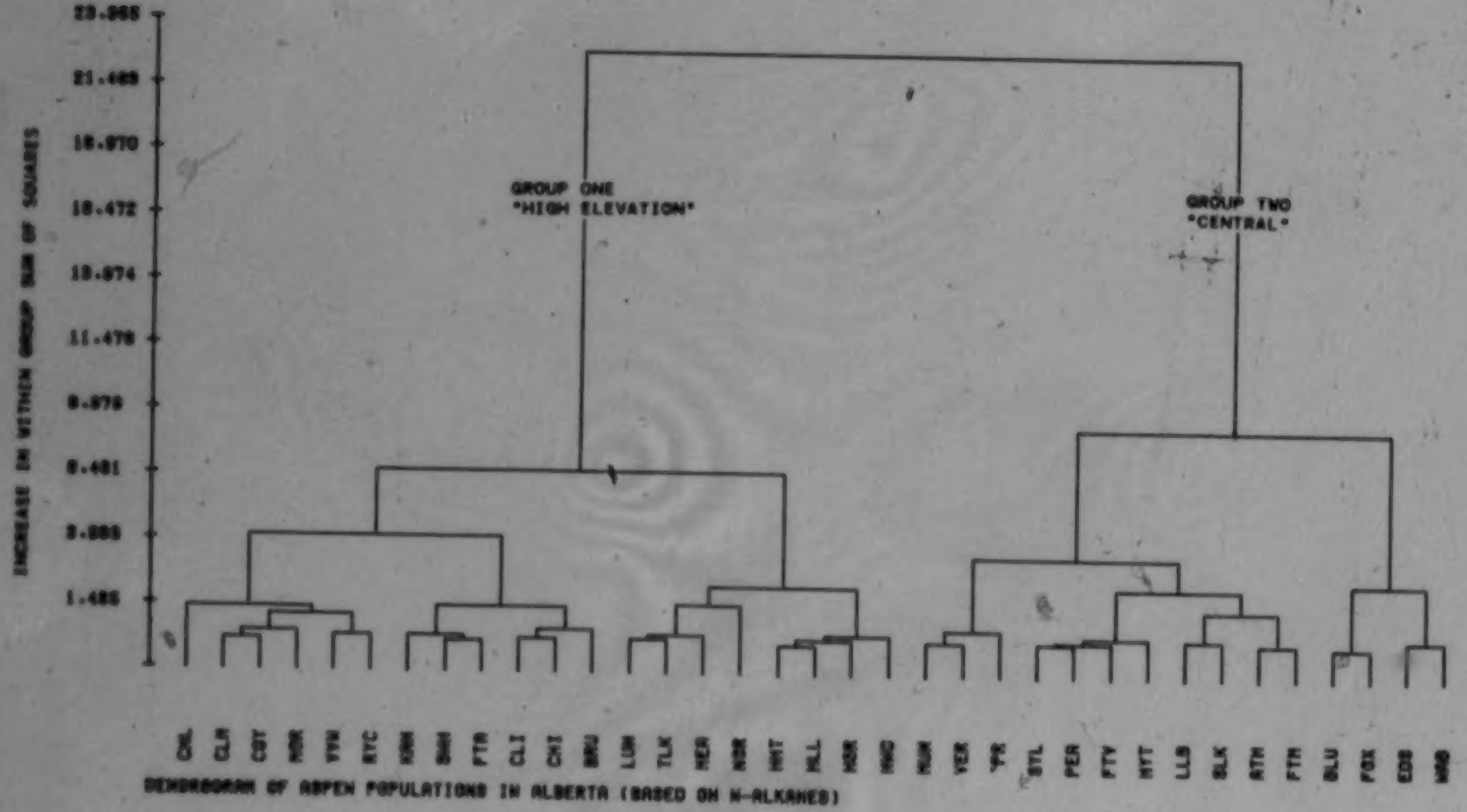

Figure 6. Dendrogram of the results of cluster analysis of 35 Alberta trembling aspen populations based on seven leaf wax alkane characters. Population descriptions are found in Table 1.

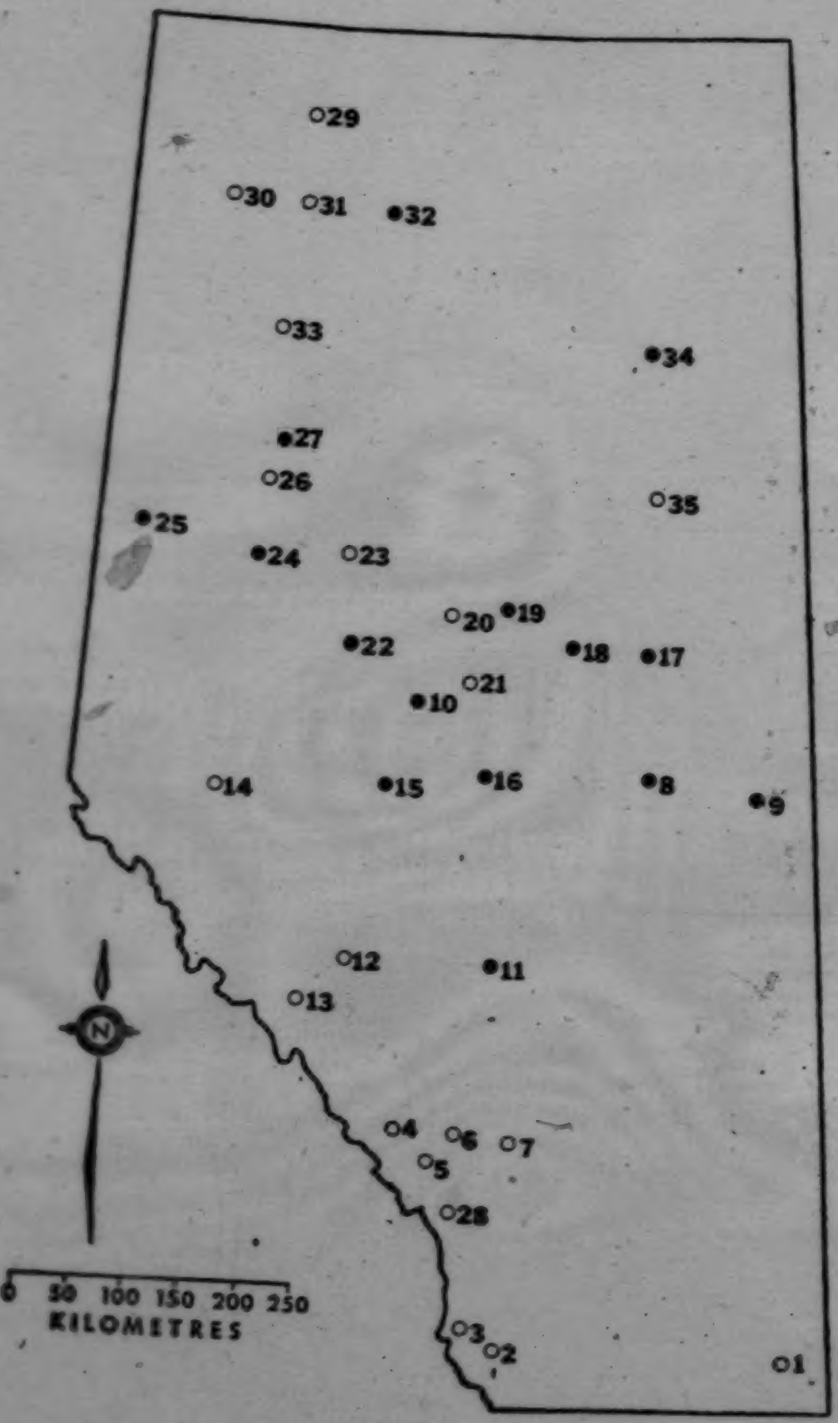
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Figure 7. Location of Alberta trembling aspen populations as grouped by cluster analysis. ○ and ● identify populations from the High Elevation and Central groups, respectively.

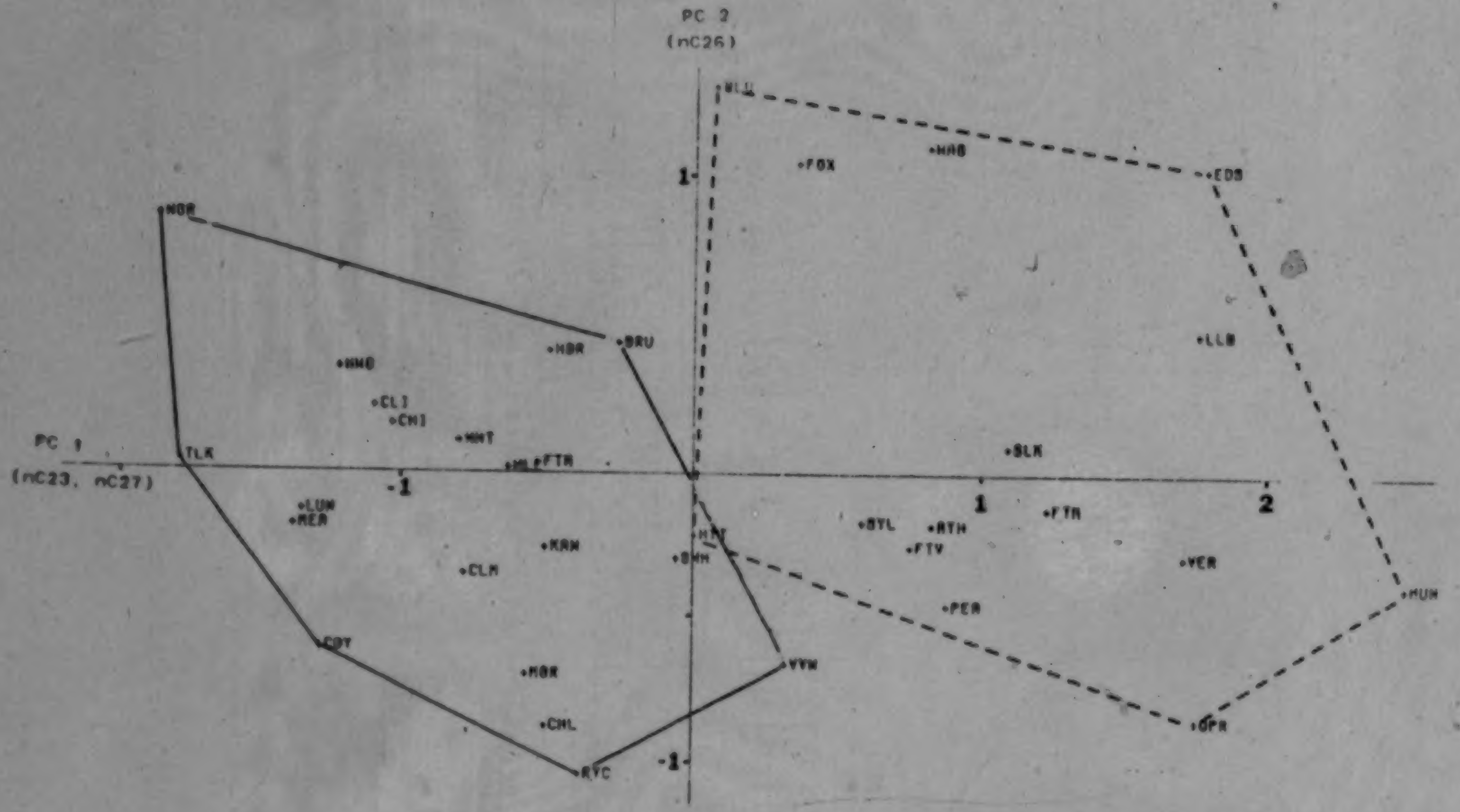


Figure 8. Ordination of 35 Alberta trembling aspen populations along the first two principal component axes. ——— and - - - represent the High and Central groups from cluster analysis, respectively. Population descriptions may be found in Table 1.

The 35 group multiple discriminant analysis extracted three significant discriminant functions (Table 17). These three functions accounted for 76 percent of the variance within the data. Variables nC25 and nC27 loaded highest on all three discriminant functions (Table 18). The two variables loaded positively on function one and negatively on functions 2 and 3.

A plot of the 35 group centroids against the first two discriminant functions indicates a partitioning of the populations in a fashion very similar to the results of the cluster and principal components analyses (Fig. 9). Population centroids from the group labelled High Elevation in the cluster analysis generally exhibited negative values along discriminant axis two, while those in the Central group exhibited positive values along discriminant axis two. Both groups were distributed along discriminant axis one. These results suggest two distinct groups of trembling aspen in Alberta, based on leaf surface wax alkanes.

Analysis of variance and discriminant analysis, using the original six input variables and groupings based on the results of the cluster analysis, indicated that the two groups could be readily characterized (Table 19). Significant differences across the two groups were found in all of the alkanes except nC26. Alkanes nC23 and nC27 exhibited the greatest between-group difference. The single extractable discriminant function was significant ($p<.001$) and was able to correctly classify 100 percent of the

Table 17. Eigenvalues, variance, and canonical correlation from discriminant analysis of 35 Alberta trembling aspen populations using six leaf wax alkanes as input variables.

FUNCTION	EIGENVALUE	CUMULATIVE VARIANCE	CANONICAL CORRELATION
1***	1.76	35.68	0.80
2***	1.17	59.28	0.73
3***	0.84	76.21	0.68
4	0.62	88.69	0.62
5	0.35	95.76	0.51
6	0.21	100.00	0.42

*** Indicates discriminant function is significant at p<.001.

Table 18. Standardized canonical discriminant function (CDF) coefficients derived from leaf surface wax alkanes from 35 trembling aspen populations in Alberta.

ALKANE CARBON NUMBER	CANONICAL DISCRIMINANT FUNCTION		
	1	2	3
22	-0.82	-0.79	-0.49
23	0.92	-0.29	-1.26
24	-0.04	0.07	-0.95
25	1.58	-2.04	-1.37
26	1.25	-0.74	-0.86
27	1.20	-2.75	-3.00

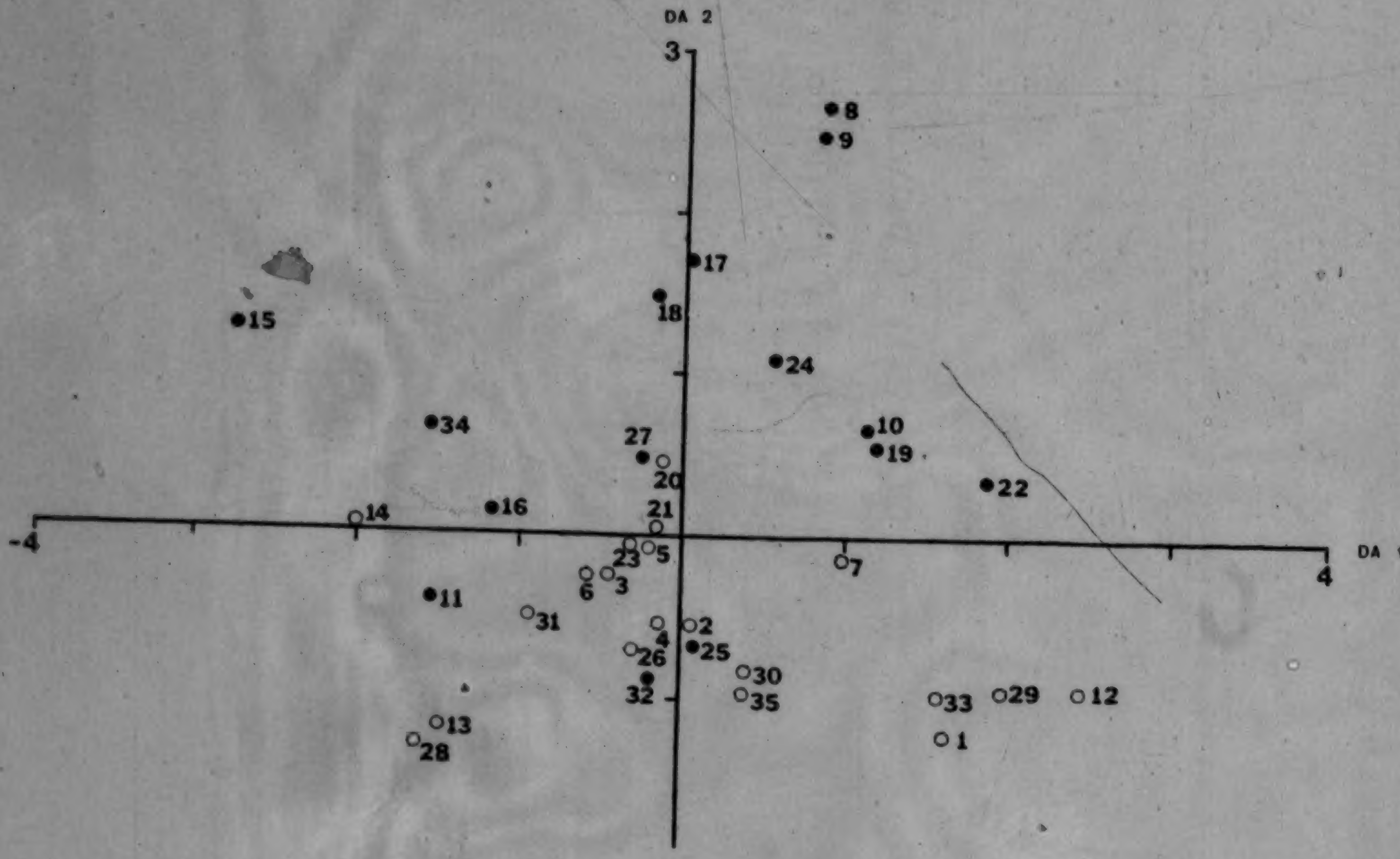


Figure 8. Plot of discriminant score centroids of 35 Alberta trembling aspen populations on first two discriminant axes. Centroids are based on six alkane variables and five clones per population. ○ and ● represent the High Elevation and Central groups, respectively.

populations to their respective group. Seventy-nine percent of the variance in the data set could be attributed to group membership. The standardized discriminant function coefficients indicate that variables nC25, nC27, and nC23 were the greatest contributors to group separation.

Geographically and environmentally, the High Elevation group is characterized by a mean elevation of 985 m ASL. an average daily temperature throughout the growing season of 11.5°C, and an average total precipitation for the growing season of 315 mm. Mean elevation for the group is downwardly biased due to the inclusion of the northern, lower elevation populations. The Central group is characterized by 650 m ASL., an average daily growing season temperature of 12.5°C, and an average precipitaion during the growing season of 300 mm.

Table 19. Summary of alkane concentration means, ANOVA F values, and standardized canonical discriminant function coefficients between the High Elevation and Central groups of Albert aspen for six leaf wax alkanes.

ALKANE	ALKANE CONCENTRATION MEANS			STANDARDIZED
	HIGH EL.	CENTRAL	F(1,33)	CDF
nC22	0.92	1.54	10.80*	2.14
nC23	5.93	9.30	52.29*	4.36
nC24	4.21	5.43	30.04*	1.87
nC25	24.80	26.85	6.66*	6.96
nC26	2.15	2.12	0.02	2.36
nC27	61.77	54.66	67.71*	6.69

* Significant at $p<.05$.

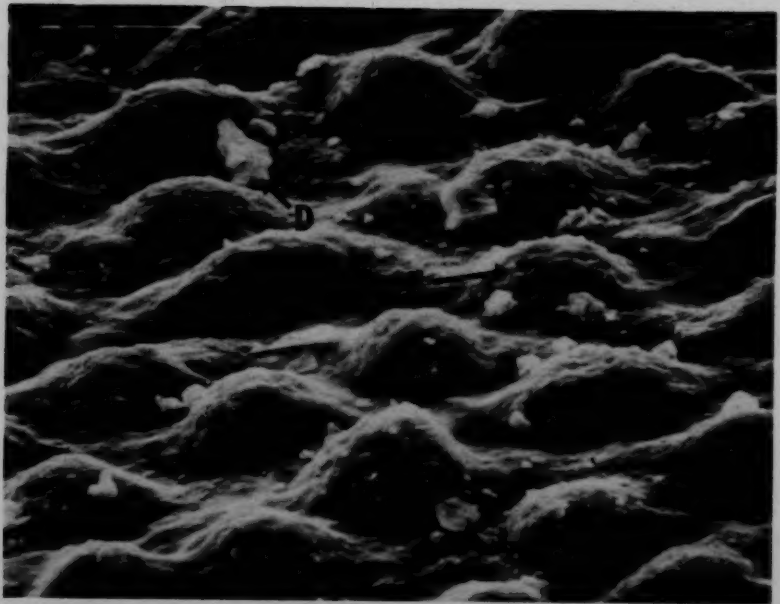
B. Scanning Electron Microscopy

Scanning electron microscopy revealed substantial amounts of surface wax present on trembling aspen foliage (Plates 1-4). When the surface wax was removed by immersing the leaves in chloroform, the upper epidermal cells appeared dome shaped (Plate 1), while the lower epidermal and subsidiary cells appeared as wavy, elongated tube-like structures (Plate 2). These abaxial structures are very similar to those found in other Populus species (Pallardy and Kozlowski 1980). Wax deposition is clearly evident among the dome-shaped epidermal cells and covering the elongated lower subsidiary cells of undisturbed adaxial and abaxial leaf surfaces (Plates 3 and 4). These surface waxes are composed of a network of cruciform-shaped platey crystals (Plate 5), although variation in wax morphology and density was observed between staminate and pistillate leaves.

At lower magnification (500X) the upper epidermal cells were not readily discernible on leaves from pistillate clones (Plate 6). In contrast, the upper epidermal cells of staminate leaves could be identified readily (Plate 7). Differences in wax morphology were apparent on the abaxial surfaces of foliage from the two sexes (Plates 8-15). At lower magnification (500X) epidermal and subsidiary cellular boundaries of pistillate leaves appear highly irregular, giving the abaxial surface a somewhat glaucous appearance (Plate 8). On staminate leaves, epidermal and subsidiary cellular boundaries appear smooth (Plate 9). These irregularities are more apparent at higher magnification (4500X - 12000X) (Plates 10-15). While waxes from leaves of both clone types exhibit a platey structure, wax crystals from pistillate leaves appear to be oriented perpendicular to the leaf surface (Plates 10-12). Alternatively, waxes on staminate leaves generally appear to be oriented horizontally to the leaf surface (Plates 13-15). This observation is particularly evident in Plate 15 where the staminate leaf sample is oriented at a 60° angle to the microscopic electron beam. A major difference in the density of the wax crystals also was apparent between the sexes. The surface wax of pistillate foliage appeared denser than that found on staminate foliage. The vertical orientation of the wax crystals, combined with this increased number of wax crystals, results in a highly irregular outer surface in pistillate foliage. Of noticeable interest throughout the scanning electron microscopic investigation was the absence of stomata on the adaxial foliar surfaces.

Plate 1 Adaxial trembling aspen leaf surface with epicuticular wax removed. "E" indicates dome-shaped upper epidermal cells. "D" dust particles. (1300X)

Plate 2 Abaxial trembling aspen leaf surface with wax removed. "G,S, and E" indicate guard cells, subsidiary cells, and epidermal cells, respectively. (2750X)


D

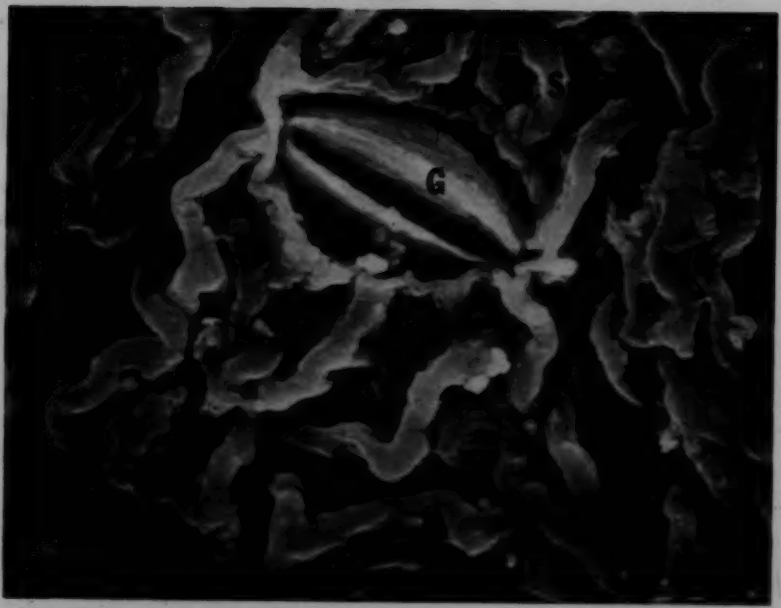

S
G

Plate 3 Adaxial trembling aspen leaf surface showing the dome-shaped epidermal cells (E) and surficial wax deposits (W). (1300X)

Plate 4 Abaxial trembling aspen leaf surface showing subsidiary cells (S), epidermal cells (E), and wax deposits (W). (1500X)

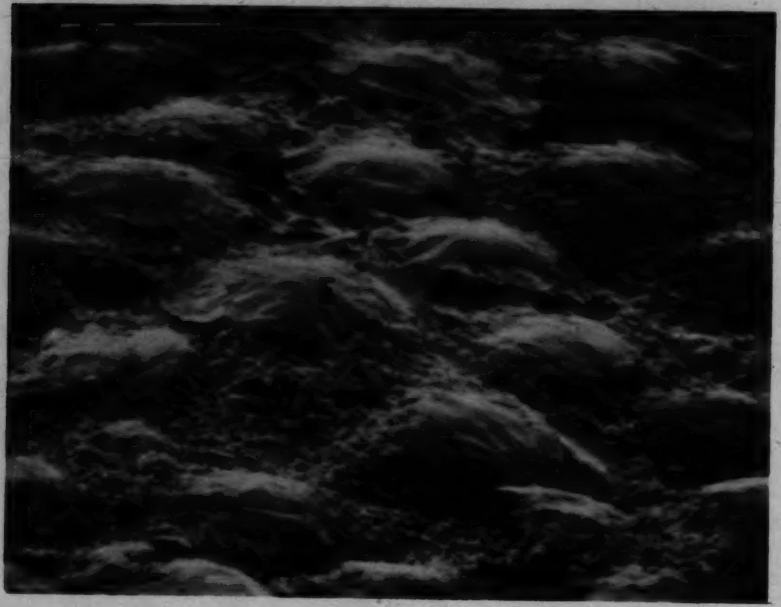


Plate 5 Abaxial trembling aspen leaf surface. Epidermal cell (E) and cruciform-shaped wax crystals (W) are evident. (12,000X)

Plate 6 Pistillate trembling aspen adaxial leaf surface. Epidermal cells (E) are not readily discernible. (500X)

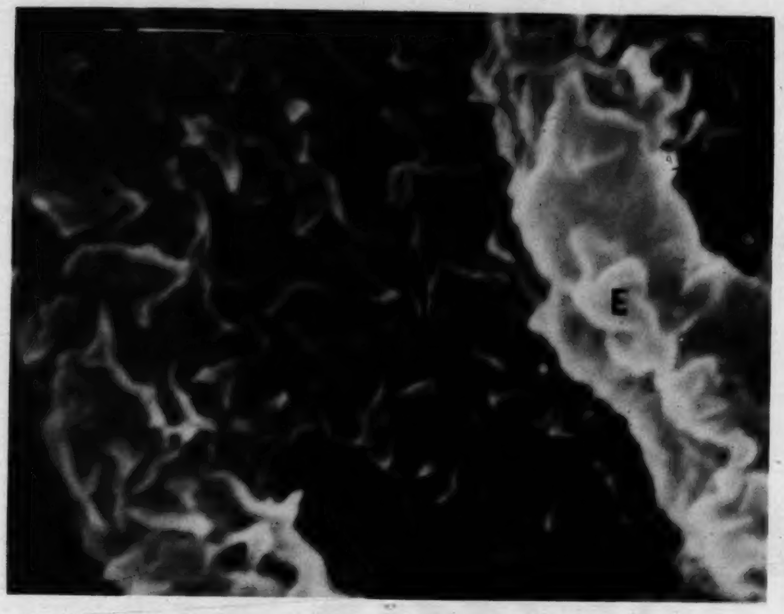

E

Plate 7   Staminate trembling aspen adaxial leaf surface. Epidermal cells (E) are easily identified. (500X)

Plate 8   Pistillate trembling aspen abaxial leaf surface showing highly irregular epidermal and subsidiary cellular boundaries (C). (500X)

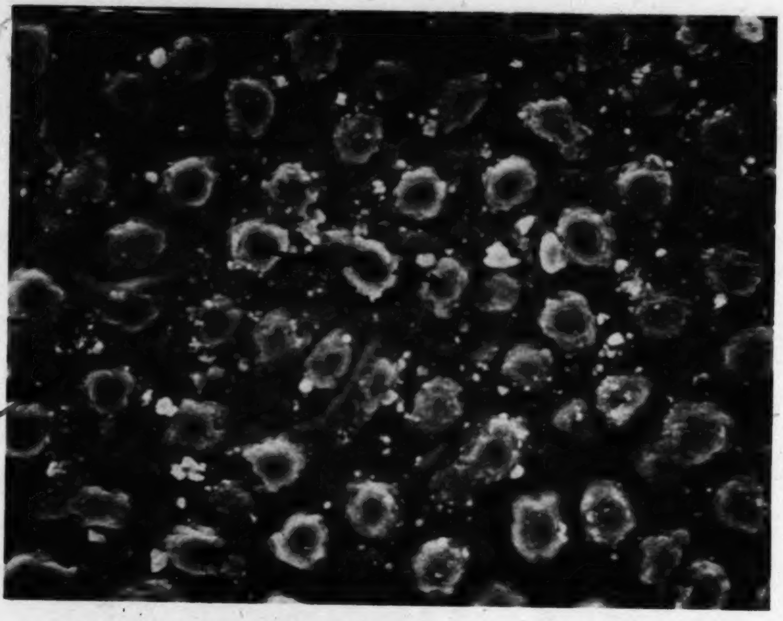
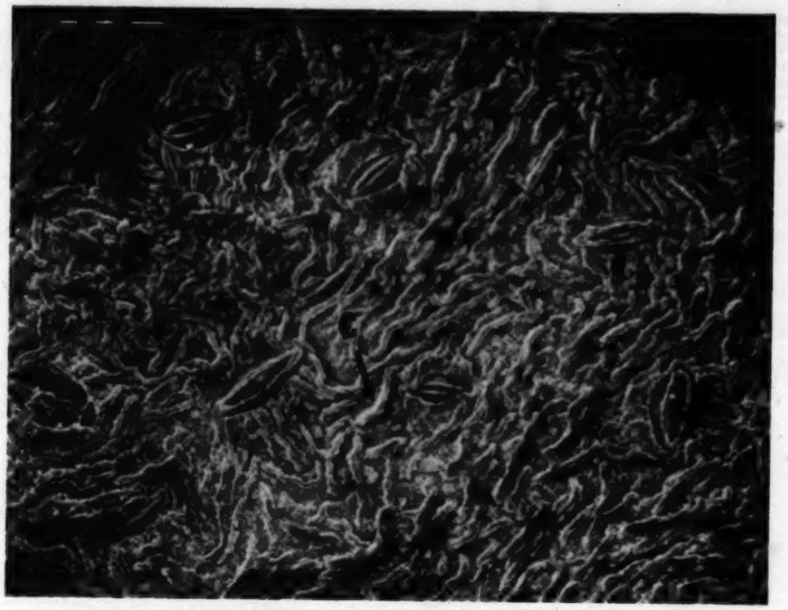


Plate 9 Abaxial staminate trembling aspen leaf surface showing relatively smooth epidermal and subsidiary cellular boundaries (C). (500X)

Plate 10 Abaxial surface of a pistillate trembling aspen leaf showing epidermal cells (E) and platey wax crystals (W) oriented perpendicular to the leaf surface. (4500X)


G

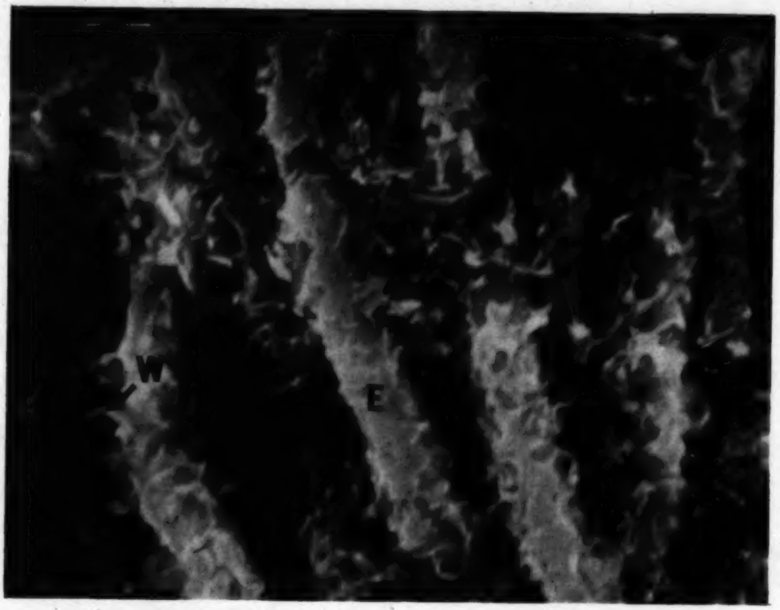
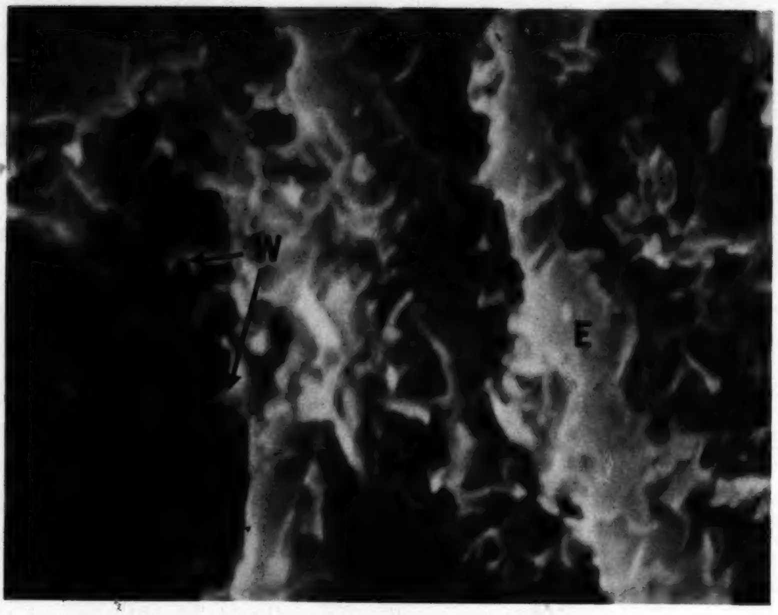

W
E

Plate 11 Abaxial surface of a pistillate trembling aspen leaf showing epidermal cells (E) and platey wax crystals (W) oriented perpendicular to the leaf surface. (6000X)

Plate 12 Abaxial surface of a pistillate trembling aspen leaf showing epidermal cells (E) and platey wax crystals (W) oriented perpendicular to the leaf surface. (11,000X)


W
E

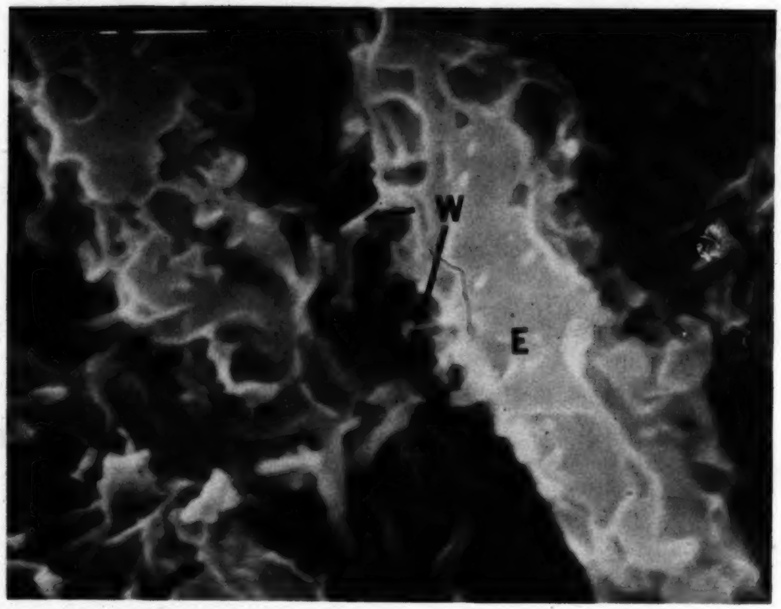
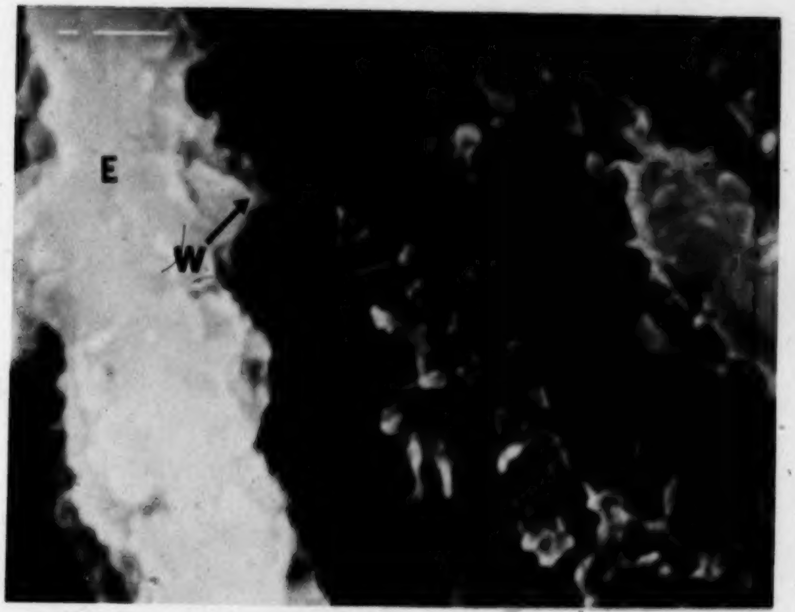

W
E

Plate 13 Abaxial surface of a staminate trembling aspen leaf showing epidermal cells (E) and platey wax crystals (W) generally oriented parallel to the leaf surface. (11,000X)

Plate 14 Abaxial surface of a staminate trembling aspen leaf showing epidermal cells (E) and platey wax crystals (W) generally oriented parallel to the leaf surface. (12,000X)


E
W

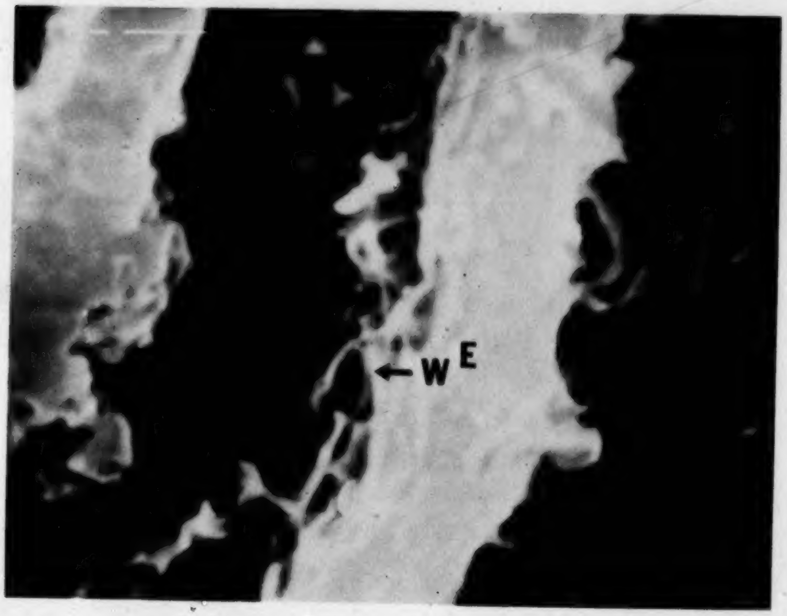
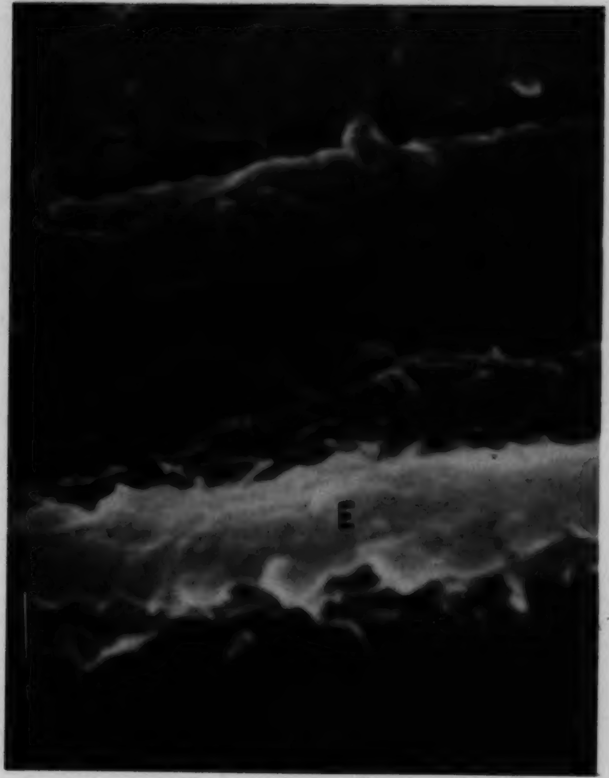

W
E

Plate 15 Abaxial surface of a staminate trembling aspen leaf. The sample is at a 60° angle to the electron beam. Epidermal cells (E) are evident. Individual horizontally oriented wax crystals (W) are visible along the leaf surface. (10,500X)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IV. DISCUSSION

Hall et al. (1965) and Chambers et al. (1976) reported leaf surface wax morphology in Eucalyptus to be closely related to wax chemical composition. Tulloch (1976) cited several additional studies where similar conclusions were drawn. These reports indicated that leaf waxes characterized by a platey morphology generally possess a relatively high proportion of hydrocarbons. The platey wax structure observed in trembling aspen foliage, therefore, suggests a relatively high proportion of hydrocarbons. Although formal analysis was not carried out, preliminary investigative GLC analyses of crude trembling aspen foliar waxes conducted prior to the present study support this speculation.

The results of the present study indicate that the hydrocarbon fraction in trembling aspen leaf epicuticular wax is composed almost entirely of long-chained, saturated, odd-carbon-numbered n-alkanes. Hadley (1980) suggested that surface waxes containing high proportions of n-alkanes provide maximum water impermeability and, as a result of the high n-alkane melting point (47 to 75°C), exhibit considerable thermal stability. Therefore, the high n-alkane content found in aspen leaf wax indicates a temperature-stable, hydrophobic outer leaf surface which should minimize evaporative losses from the plant. This adaptation, combined with the absence of adaxial stomata, would be highly beneficial to plants such as trembling aspen in the Rocky Mountains or at the edge of the prairie that are periodically subjected to high temperatures and drought.

The alkane profile of trembling aspen is similar to the alkane profile reported for *P. tremula* L.; however, it is quite distinct from those of *P. nigra* var *italica* (Puroi) Dost., *Salix caprea* L., and *S. babylonica* L. (Stransky *et al*. 1967). In *P. tremula*, alkanes nC27, nC25, and nC23 comprised approximately 45,35, and 18 percent, respectively, of the wax alkane fraction. These same alkanes comprised approximately 55, 24, and 10 percent of the wax alkanes of trembling aspen. The alkane profile of *P. nigra* var. *italica*, relative to those of the two *Leuce* poplars, indicated a greater concentration of nC29 (35 vs. 5 percent) and the presence of alkanes nC30 and nC31. Intergeneric alkane differences between *Salix* and *Populus* are apparent, since both *Salix* species contained considerably greater concentrations of nC27. *S. caprea* was characterized by the absence of even-carbon-numbered compounds.

Quantitative leaf wax alkane analysis, therefore, appears to demonstrate chemotaxonomic utility within the section *Leuce*, among the various sections of *Populus*, and between *Populus* and *Salix*. These observations also signify a close biochemical relationship between the two *Leuce* poplars and a biochemical dissimilarity between the *Leuce* and *Aigeros* poplars. Steele and Ronald (1973) reached a similar conclusion following GLC analysis of bark phenolic compounds from *Leuce*, *Aigeros*, and *Tacamahaca* poplars. Biochemical analysis of the leaf wax alkane and bark phenolic compounds, therefore, appears to support the present section subdivision, which is based on morphological and anatomical criteria.

Temporal Variation

The significant mid-June transition which occurred in all of the alkanes except nC23 and nC25 may be a function of foliage development and ageing. Faboya et al. (1980) reported leaf wax alkane concentrations in three Khaya A. Juss. species varied with leaf age. In Khaya, the major transition in alkane composition occurred during a three to six week period following leaf flush. In the 27 succeeding weeks, no further variation in alkane composition was noted. They attributed the transition to leaf ageing. The temporal pattern of leaf wax alkane variability in Khaya is strikingly similar to that observed in trembling aspen; however, in trembling aspen the major transition occurred approximately 4-6 weeks following leaf flush. These results suggest a close relationship between leaf expansion and wax development. Such a relationship has been reported in Eucalyptus (Hallam 1970) and Picea (Reicosky and Hanover 1976). Hallam reported that following removal of the surface wax from Eucalyptus foliage, wax regeneration took place rapidly on leaves which were expanding, while wax regeneration on fully expanded leaves was extremely slow. The conclusion was that Eucalyptus foliar surface waxes are deposited primarily in the leaf expansion phase. Reicosky and Hanover used scanning electron microscopy to describe the seasonal development of surface waxes of Picea pungens (Engelm.) and found wax deposition continued throughout leaf expansion and reached a stable maximum approximately two months following leaf flush. If these patterns of wax development are common among plant species, the patterns of temporal variation found in aspen and Khaya leaf wax alkanes suggest there may be a relationship between leaf wax deposition and wax chemical composition. In trembling aspen it appears that surface waxes deposited within the first two weeks of leaf flush are composed primarily of alkanes nC25 and nC27, whereas surface waxes deposited within the subsequent two to three weeks are composed primarily of the remaining five alkanes. By approximately the sixth week of leaf expansion, the alkane composition of aspen leaf wax has stabilized and remains uniform throughout the growing season. This period of stabilization may coincide with the termination of leaf surface wax deposition. One may speculate that a pattern of wax deposition similar to that suggested for aspen also may be responsible for the temporal alkane variation in Khaya.

Temporal variation also may explain the variability in alkane concentrations between leaves from long and short shoots. On long shoots, which continuously produce new foliage until vegetative bud set, substantial ontogenic variation would be found among leaves. This would imply substantial variability in epicuticular wax development. Therefore, while the surface waxes of leaves from short shoots would have been stable at the time of collection, the waxes from many of the long-shoot leaves may have been in various stages of development.

Temporal variation, in any event, must be considered if leaf wax alkanes are to be used in chemotaxanomic or genecological investigations of trembling aspen. The optimum period for the initiation of collections of aspen foliage for alkane analysis appears to be the middle of the growing season following surface wax stabilization.

Inter- and Intraclonal Variation

Since environmental conditions over the collection site appeared relatively uniform, one may speculate that the significant among-genotype differences in all seven alkanes is the result of genetically controlled differences among the clones. This premise is substantiated by the relatively high broad-sense heritability estimates. These estimates, which apply solely to the clones investigated in this experiment, indicate the majority of the phenotypic variance in aspen leaf wax alkanes is attributed to genetic differences among clones. Further research, incorporating a greater number of clones and research sites, would be required to determine the accuracy of the broad-sense heritabilities estimated in the present study.

Van Buijtenen *et al*. (1959) reported broad-sense heritabilities for six leaf morphological characters of aspen ranged from 0.29 to 0.64. Many of these leaf characters, such as leaf shape, base angle, and number of serrations, have proven useful in the delineation of aspen clones (Barnes 1969). Since the alkane hertability estimates are higher than those reported for leaf morphological characters, leaf wax alkane characters appear to be less modified by the environment than morphological characters. The notable exception to this is nC25, which appears to undergo substantial environmental modification. With the exception of nC25, leaf wax alkanes appear to be useful clone delineators. This postulation is supported by the results of the interclonal analysis of variance and the heritability estimates, which indicate significant interclonal and limited intraclonal variation, respectively. Of the seven alkanes present in aspen leaf wax, compounds nC23 and nC27 would probably serve as the most accurate clone characterizers, since these compounds are present in the greatest concentrations and exhibit limited within-clone variability. These alkanes probably could be used either individually or in combination with other alkanes to discriminate among clones. However, as when using morphological characters to delineate clones, inherent problems would be encountered when using leaf wax alkanes to delineate clones.

Although no two clones exhibited identical alkane profiles, identification of individual clones from a large number of clones, solely on the basis of alkane profiles, would be difficult. This difficulty arises from the fact that differences in alkane concentrations among several of the clones were so slight that discrimination among those clones would be difficult. The limited intraclonal variation compounds this problem. Therefore, other delineation criteria would have to complement alkane analysis when discriminating among large numbers of aspen clones. If prescreening revealed unique alkane profiles among a small number of clones, the leaf wax alkanes might be used to accurately distinguish among the clones and identify ramets within a clone. Foliage for this type of analysis would have to be collected from short shoots in a standardized crown stratum to eliminate error introduced by within-crown variation. Collection within the stratum could be in any direction since direction within crown has little influence on the alkane concentrations.

The inverse relationship between nC27 concentration and height within the crown, and the positive relationships betwen nC23 and nC25 and height within the crown, parallel the relationships between the three alkanes and average growing season temperature. This similarity can be attributed to maximum temperatures found in the upper tree crowns (Geiger 1966). This variability in alkane concentration appears to result in variation in leaf surface wax morphology. Reduced concentrations of nC27 and increased concentrations of nC23 and nC25 in both the upper crown and the geographic "Central" group appears to produce wax crystals oriented perpendicularly to the leaf surface (Plates 10-12). This perpendicular wax orientation, combined with the strongly undulating abaxial cuticle, results in a somewhat glaucous abaxial leaf surface. This type of leaf surface has been shown to be an adaptation to hot environments in Eucalyptus camaldulensis Dehn. (Karshon and Pinchas 1971), and may serve a similar function in trembling aspen.

Glaucousness in leaves tends to increase light reflectance and reduce potentially harmful leaf temperature buildup (Karschon and Pinchas 1971; Reicosky and Hanover 1978). Along with the protection afforded the leaf from thermal damage, reduced leaf temperature would reduce the vapour pressure gradient across the leaf, thereby reducing water losses from the plant (Hadley 1980). The irregular wax surface, combined with the undulating abaxial leaf surface in aspen, also may disrupt air flow under the leaf surface, which could reduce plant water losses. These adaptations, which obviously would be advantageous to foliage in the upper tree crowns and within the warmer environments of the Central group, may have a genetic basis, since wax morphology has been shown to be under genetic control (Barber and Jackson 1957; Lundqvist et al. 1968; von Wettstein-Knowles 1972).

Sexual Variation

Statistical analyses of the alkane data and the results of scanning electron microscopy indicate fairly distinct biochemical and morphological differences between leaf surface waxes of staminate and pistillate trembling aspen clones. These biochemical differences would have to be accounted for in any trembling aspen chemotaxonomic or genecological investigations. Discriminant analysis of leaf wax alkane characters appears to provide a satisfactory statistical means for discriminating between the two sexes. Once derived, the discriminant function may be used to predict, with relatively high accuracy, the gender of aspen clones on a particular site.

Blake *et al*. (1960), using paper chromatography on vegetative bud extracts from 20 aspen clones, reported biochemical differences between staminate and pistillate clones. This conclusion was later retracted when the technique was applied to a larger number of clones (Blake *et al*. 1967). Although only 35 clones were used in the present study, the precise GLC analytical technique, the replication of genotypes sampled, the consistent sampling of short-shoot leaves from a standardized crown position, and the relationship between leaf wax morphology and chemical composition justify the conclusion of biochemical differences between sexes. This evidence of biochemical differences between sexes in trembling aspen supports the findings of Mitton and Grant (1980) who reported significantly different allelic frequencies between male and female aspen at the phosphohexose isomerase gene locus. These differences in leaf wax chemical composition and morphology also may be adaptively significant to the species.

If trembling aspen populations in Alberta exhibit a pattern of sexual spatial segregation similar to that reported in other dioecious species (Freeman et al. 1976; Grant and Mitton 1979; Il'in 1973; Opler and Bawa 1978; and Putwain and Harper 1972), one would expect similar sexual niche occupation. That is, pistillate clones should predominate in the warmer, low elevation areas and staminate clones should predominate in the cooler, high elevation areas. Since Grant and Mitton (1979) found this pattern of spatial segregation in Colorado aspen, it would be reasonable to expect similar sexual sorting in Alberta aspen, which also exists over a wide range of elevations.

Of the 35 local populations sampled, the difference in average elevation between the two groups delineated by multivariate analyses of the alkane characters was 335 m; therefore, on a macro-geographic scale, one would expect pistillate clones to predominate in the lower, Central group, and staminate clones to predominate in the High Elevation group. One might also expect extreme localized sorting of sexes should sufficient elevational or moisture gradients exist. Regardless of the mode of sex determination in aspen, this type of segregation is no doubt a sexual reproductive strategy of the species.

Flowering in trembling aspen typically occurs several weeks prior to leaf flush, with pistillate clones usually flowering ahead of staminate clones (Einspahr and Winton 1976). Pistillate clones, because of their prolonged reproductive effort, must channel more resources into reproduction, often during the driest part of the year. Natural selection, therefore, would probably favour femaleness in the low elevation sites where temperatures are higher, the growing season longer, and moisture deficits at critical stages of sexual reproduction are reduced. Alternatively, staminate clones expend very little energy in sexual reproduction as anthesis usually occurs approximately two weeks following flowering and generally prior to leaf flush. As a result of this minimal reproductive effort and extremely short reproductive event, pollen production is not likely to be greatly affected by the environment. Staminate clones likely would not suffer, in terms of sexual reproductive capacity on the higher, drier sites, which exhibit a shorter growing season. Such a reproductive strategy appears not uncommon among temperate, dioecious plant species (Charnov and Bull 1977; and Freeman _et al_. 1976).

Adaptations improving plant water relations during the sexual reproductive period would, no doubt, be highly advantageous to a species. Genes controlling such a trait would likely be selected positively, particularly in seed-producing plants exhibiting prolonged seed development. An irregular leaf surface produced by epicuticular waxes could be an example of such a favourable moisture conserving adaptation. In trembling aspen, however, the majority of the sexual reproduction event is completed prior to leaf flush. Only in seed development is there a short overlap between vegetative and reproductive development. Therefore, leaf surface waxes no doubt have minimal effect on the sexual reproductive capacity of trembling aspen; however, the waxes would undoubtedly influence the plant's water relations and photosythetic capability during the vegetative growth phase. Thus, the leaf epicuticular waxes may contribute indirectly to sexual sorting in trembling aspen.

By mechanisms previously described, an irregular leaf surface would reduce plant water losses and protect the foliage from thermal damage during hot summer periods. Therefore, the highly irregular leaf surface of pistillate clones may be an ecological adaptation to hotter environments. At higher elevations, where temperatures are lower, one may speculate that leaf temperature buildup would not be a serious problem. Since glaucousness in Eucalyptus has been shown to increase light reflectance and decrease photosynthesis (Cameron 1970), one could speculate that the increased density and perpendicular orientation of pistillate trembling aspen leaf epicuticular waxes probably results in a lower rate of photosynthesis in pistillate clones, than in staminate clones. This hypothesis is supported by the work of Bordeau (1958), who reported significantly lower respiration rates in staminate aspen and lower net photosynthesis in pistillate aspen. This reduced photosynthetic rate in pistillate plants may be a function of either leaf wax quantity or wax morphology, and would undoubtedly be disadvantageous at higher elevations. Selection in cooler environments would favour the non-glaucous phenotypes which exhibit higher rates of photosynthesis. In trembling aspen these non-glaucous phenotypes appear to be primarily staminate clones.

From these observations it may be inferred that spatial segregation is probably a reproductive strategy in trembling aspen, and that selection favours pistillate clones in the warmer, low elevation sites. However, to successfully occupy these sites, pistillate clones require mechanisms to control leaf temperature buildup and water losses. It appears the leaf epicuticular waxes may provide such a mechanism. In the cooler, high elevation areas, selection appears to favour staminate trembling aspen clones which, as a result of less dense and horizontally oriented leaf wax crystals, photosynthesize at a higher rate than pistillate clones. Considered jointly, these factors support the postulation that the leaf epicuticular waxes of aspen indirectly contribute to sexual spatial segregation within the species.

Geographic Variation

The results of the analysis of the geographic data indicate that trembling aspen exhibits substantial interpopulation variation in epicuticular leaf wax alkane composition. This high level of phenotypic variation is not surprising considering the variety of ecologically diverse sites occupied by trembling aspen in Alberta.

The pattern of phenotypic variation found in the leaf wax alkanes of trembling aspen in Alberta appears closely related to the environment, particularly temperature and precipitation. This relationship is not unusual, since it is well documented that variation in natural plant populations is associated with environmental gradients (e.g., Barber 1955; Barber and Jackson 1957; Barnes 1975; Hamrick and Allard 1972; Heslop-Harrison 1964; Mitton et al. 1977). The strongest relationship among the alkane, geographic, and environmental variables was between the three predominant alkanes and mean growing season temperature. Moderate relationships were found between mean growing season precipitation and three of the seven alkanes. Of the geographic variables, elevation exhibited the strongest relationship with the alkanes. The congruent relationships exhibited between the alkanes and the parameters of elevation and mean growing season temperature are to be expected since elevation and temperature are strongly correlated.

The multivariate analysis supported the results of the univariate ANOVA and regression analyses by delineating natural groupings of aspen populations on the basis of similar leaf wax alkane consititution. The two groups delineated by cluster and discriminant analyses are easily characterized on the basis of environmental criteria. The Central group has a lower mean elevation, a mean growing season temperature approximately 1C° higher, and receives slightly less precipitation during the growing season than the High Elevation group. Populations in the Central group exhibit a lower proportion of alkane nC27 and a higher proportion of alkanes nC23 and nC25 than populations in the High Elevation group. This pattern of variation parallels the pattern of alkane variation found within the tree crown and substantiates the conclusion of an inverse relationship between nC27 and temperature, and a positive relationship between both nC23 and nC25 and temperature.

From the data it is impossible to accurately determine the basis for the observed pattern of alkane geographic variation. Genetic differences or phenotypic plasticity may be responsible for the observed phenotypic variation. Based on the work of Barber (1955), Barber and Jackson (1957), and Lundqvist et al. (1968), all of whom suggest a strong relationship between phenotypic and genotypic differences in surface wax morphology, one may attribute a major portion of the observed variation to genetic differences among the populations. This conclusion assumes a relationship between chemical composition and wax morphology (Chambers et al. 1976; Jeffree et al. 1975). To substantiate this conclusion, a common garden experiment would be required to reduce the confounding affects of the environment. Phenotypic plasticity may be effectively eliminated as a possible mechanism responsible for the observed variation, since environmental factors tend to modify the quantity of wax produced and not wax morphology (Banks and Whitecross 1971).

Since a reduced concentration of nC27 and increased concentrations of nC27 and nC25 appear to produce wax crystals oriented perpendicular to the surface of aspen leaves, the Central group would exhibit a highly irregular leaf surface. As previously mentioned, this type of leaf surface would reduce leaf temperatures and plant water losses. These plants, therefore, would be better adapted for hotter, drier environments. Natural selection would favour genes controlling this type of wax morphology in such an environment. In the High Elevation group, genes producing wax crystals oriented horizontally to the leaf surface likely would be favoured as this type of leaf wax morphology decreases light reflectance and increases rates of photosynthesis.

The clinal variation that aspen leaf wax alkanes and, presumably, wax morphology exhibit with elevation is similar, though inverse, to the clinal variation of leaf glaucousness in Eucalyptus urnigera (Barber and Jackson 1957). These authors attributed clinal variation in glaucousness to natural selection, and suggested that in regions of great ecological diversity a simultaneous clinal change in the frequency of genes at a number of loci may be expected. As in the Eucalytus species, natural selection may be maintaining a cline of genes controlling leaf wax chemical composition and, therefore, wax morphology in populations of trembling aspen in Alberta.

V. CONCLUSIONS

Several conclusions regarding the leaf epicuticular wax chemical constitution and morphology of _Populus_ _tremuloides_ Michx. may be drawn from the present study. These conclusions may be summarized as follows:

1. Epicuticular waxes of trembling aspen appear to be composed primarily of n-alkanes. The hydrophobic nature of epicuticular wax composed of n-alkanes makes trembling aspen ecologically adapted to relatively warm, dry environments. As in the majority of plant species, odd-carbon-numbered alkanes predominate. Alkane nC27 is the predominant compound, comprising approximately 55 percent of the alkane fraction.
2. A considerable amount of phenotypic variability exists in trembling aspen leaf wax alkane concentrations. The majority of this variation appears to have a genetic basis. Therefore, the alkanes possess considerable utility for future chemotaxonomic and genecological investigations within the species. Concentrations of alkanes nC23, nC25, and nC27 may be used either individually or in combination as clone delineators or to discriminate between aspen and other _Populus_ species.
3. A major transition in aspen leaf wax alkane composition occurs after approximately six weeks of leaf expansion. This transition may be attributed to surficial wax deposition occurring during the leaf expansion phase.

The alkanes remain relatively stable following this transition period.

4. Significant differences in alkane concentrations exist among clones; among lower, middle, and upper crown positions; and between shoot types. Genetic differences appear to be responsible for much of this variation.
5. Significant differences in alkane concentrations exist between staminate and pistillate aspen leaf waxes. Staminate foliage, relative to pistillate foliage, contains higher concentrations of nC23 and nC25, and lower concentrations of nC27. Discriminant analysis may be used to derive a discriminant function that will identify, with relatively high accuracy, the sex of aspen clones on a particular site.
6. Differences in alkane concentrations appear to result in variation in leaf epicuticular wax morphology between the two sexes. Pistillate foliar wax is composed of a dense array of cruciform-shaped, platey wax crystals which are oriented perpendicularly to the leaf surface, while staminate foliar waxes are less dense and are oriented horizontally to the leaf surface. This variation in foliar wax could give pistillate clones the selective advantage in hotter environments and staminate clones the selective advantage in cooler environments.
7. Significant phenotypic variation in leaf wax alkane composition exists among trembling aspen populations in Alberta. Significant relationships exist betwen leaf wax

alkane composition and both average growing season daily temperature and elevation. In cooler high elevation regions, aspen leaf waxes contain significantly lower concentrations of nC22, nC23, nC24, and nC25, and higher concentrations of nC27 than aspen leaf waxes from populations found in warmer, low elevation regions.

VI. FUTURE STUDIES

The present work provided information pertaining to temporal, inter- and intra-clonal, sexual, geographic, and morphological variability in the leaf epicuticular wax alkanes of trembling aspen. The major contribution of the study was in obtaining a comprehensive understanding of trembling aspen leaf wax chemical composition and morphology, and in furthering our understanding the inter-and intraspecific variability of secondary metabolic compounds. A logical extension of this research would be as follows:

1. Use scanning electron microscopy to monitor the temporal development of aspen leaf waxes, to further investigate the morphological differences between gender, and to investigate wax morphology from many ecologically diverse aspen populations.
2. Conduct a common garden experiment to assess the extent of the variability in genes controlling wax morphology.
3. Conduct controlled environment experiments to evaluate the extent of genotype-environment interaction exhibited by aspen leaf waxes.
4. Perform controlled crosses among genotypes from a variety of habitats to study the genetic control of the leaf epicuticular wax alkanes.

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